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FONDAZIONE GHISLIERI

***Progetto "Progressi in Biologia e Medicina"***

*18° Corso di formazione avanzata*

# Immunoterapia

22-24 maggio 2019, Collegio Ghislieri, Pavia

*A cura di CarloAlberto Redi*

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# Indice

# Prefazione

L'assegnazione del premio Nobel per la fisiologia o la medicina del 2018 a James P. Allison e al giapponese Tasuku Honjo "per la loro scoperta della terapia del cancro attraverso l'inibizione della regolazione immunitaria negativa" giunge al termine di un percorso che ha visto molti ricercatori impegnati "ad istruire il sistema immunitario per riconoscere ed eliminare i tumori", biologi e medici che hanno sviluppato quella che ad oggi ha tutte le caratteristiche e lo statuto epistemologico per essere a tutti gli effetti una nuova disciplina: l'immunoterapia oncologica. E questa costituisce anche un nuovo approccio che ha già trasformato radicalmente il trattamento clinico di alcune forme tumorali.

L'ingegnerizzazione dei linfociti T ha segnato l'inizio di questa nuova era in biologia e medicina, dopo che per più di un secolo gli sforzi della ricerca si erano focalizzati sull'amplificazione dei meccanismi alla base dell'attivazione immunologica, giusto quelli impiegati dal sistema immunitario per eliminare virus e batteri.

Si è così affacciato e costituito un potente paradigma concettuale basato sull'impiego di cellule quali piattaforma terapeutica: la manipolazione di cellule viventi ed il loro impiego, che è cosa ben diversa dall'utilizzo di anticorpi o di piccole molecole. Le cellule T – CAR (Chimeric Antigen Receptor) hanno dimostrato che cellule ingegnerizzate del sistema immunitario possono essere utilizzate come una nuova potente classe di strumenti terapeutici anti-cancro. L'esperienza clinica ha poi aiutato a definire quelli che possiamo considerare i criteri di sicurezza che debbono essere raggiunti per rendere la terapia con le cellule T ingegnerizzate efficace contro una vasta gamma di tumori. Le recenti possibilità tecniche offerte dalla "Biologia Sintetica" per ingegnerizzare in modi del tutto nuovi le cellule del sistema immunitario promettono di espandere nel breve volgere temporale i campi di applicazione dell'immuno-oncologia.

L'immunoterapia oncologica è dunque ora una disciplina che attende di veder affermarsi nuovi avanzamenti del proprio corpo di sapere, in primis nel trattamento dei tumori solidi.

I docenti del corso IMMUNOTERAPIA presentano i risultati consolidati e discutono di sicurezza, affidabilità ed efficacia di nuove proposte immunoterapeutiche, in diversi campi di loro pertinenza e nei quali sono considerati figure di riferimento.

Nell'insieme lo sforzo didattico di tutti i relatori (ai quali va uno speciale ringraziamento) ha permesso di produrre il volume degli atti che certamente aiuterà a rafforzare quanto in aula verrà presentato e discusso.

Un grazie particolare all'Amministrazione del Collegio che organizza i nostri corsi ed a tutte le persone che con grande competenza professionale (e pazienza!) ne permettono la realizzazione.

*CarloAlberto Redi*

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# 1

# Antibodies: from antitoxin to magic bullets

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No area of medical research has had a greater impact on human health than the study of immunity. Immunity, namely the ability of an organism to fight and survive infection, is the outcome of the concerted activity of a number of cell types, namely lymphocytes, macrophages and dendritic cells, which cooperate in recognising and attacking viruses, bacteria or parasites able to invade the body and potentially cause disease. The immune reponse of vertebrate animals operates in two ways: in a first mechanism certain subpopulations of lymphocytes (cytotoxoc T lypmphocytes and natural killer (NK) lymphocytes) attack and kill body cells damaged by the pathogens. This action is very effective in 'damage limitation', ie in containing the ability of the pathogen to survive and propagate in the host. A second and major mechanism of immunity involves the production of soluble proteins that bind specifically to the pathogen and lead to (i) neutralisation of viral particles or bacterial toxins, (ii) bacterial lysis or, (iii) enhanced bacterial phagocytosis. The vaste therapeutic potential of antibodies was immediately apparent right from start when antibodies were discovered in the last decade of the 19th century but nearly a century elapsed before an effective methodology was discovered in 1975 that enabled the production of homogenous population of antibodies (monoclonal antibodies). This lecture will discuss the technologies currently available for the production of monoclonal antibodies and will offer selected examples of therapeutic applications of monoclonal antibodies in infectious diseases, chronic inflammation, autoimmunity and cancer.

## The path to human monoclonal antibodies

Antibodies were discovered by Emil A von Behring and Shibasaburo Kitasato in Berlin in 1890 as serum substance(s) able to protect animals and humans from the potent and typically lethal activity of diphteria toxin (1). The discovery rapidly led to 'serum therapy', namely the injection of horse serum containing toxin-neutralising antibodies in diphteria patients and this practice had a major impact in diphteria control. In subsequent years serum therapy was extended to other infectious diseases (gas gangrene, botulism and tetanus) but the use of serum hindered the full potential of antibody-based therapies because serum contains hundreds of proteins and not just antibodies and because serum antibodies are complex mixtures in which the species is often a minor component, whose activity is often obscured by antibodies of other specificity.



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In 1975 Cesar Milstein e George Kohler changed all this by discovering a method for immortalising and cloning the antibody-producing cells: the B lymphocytes (2). The discovery of 'monoclonal antibodies' heralded a new era in antibody-based diagnosis and opened new prospects for antibody therapies. The latter, however, required further and extensive research before antibodies could be widely employed in therapy.

The strategy that enabled cC Milstein and G Kohler to immortalise B lymphocytes exploited somatic cell hybridisation, namely the fusion of B lymphocytes with immortal derived from B lymphocyte tumours (myeloma). This outstanding discovery, however, met with an unexpectd problem, the instability of human B lymphocyte - myeloma hybrids and, as a consequence, a major obstacle in the production of human monoclonal antibodies for therapy (human monoclonal antibodies are needed for therapy because the use of mouse or rat monoclonal antibodies leads to an anti-antibody reactions in patients as the the rodent proteins are recognised as foreign by the human immune system).

The production of human monoclonal antibodies has been successfully accomplished in four different ways in the course of the last few decades. In a first approach protein engineering has been employed in order to 'humanise' rodent antibodies. This approach required:

1) cloning the antibody heavy and light chain genes from a mouse or rat hybrid myeloma line secreting the antibody of the desired therapeutic activity;
2) loning antibody heavy (H) and light (L) genes from a human myeloma line and;
3) fusion - at the DNA level - of rodent and human sequences in order to generate a so-called chimaeric antibody (a protein containing the VH and VL domains of the rodent antibody) (3) or a so-called humanised antibody (a protein only containing short patches of the rodent antibody sequences sufficient to confer antigen-specificity and biological activity (4). Both chimaeric and humanised rodent antibodies are much less immunogenic than their rodent counterpart when introduced into patients and are thus endowed with higher therapeutic activity as a result of their longer life.

In a second strategy, human monoclonal antibodies can be produced in mice in which the loci encoding endogenous (murine) antibody chains have been inactivated and in which the genes encoding human antibodies have been introduced by transgenesis. The consequence of this extensive genomic engineering is that these knock-out/transgenic mice, when challenged with antigen, can only produce human antibodies, which can be immortalised effectively with the somatic cell hybridisation technology devised by C Milstein and G Kohler (5, 6).

A third strategy currently in use for production of human monoclonal antibodies exploits the ability of the Epstein-Barr virus (EBV) to immortalise human B lymphocytes. EBV is a complex DNA virus (its genome encodes 85 gene) and does infect B lymphocytes and certain types of epithelial cells. The ability of EBV to immortalise B lymphocytes has been known for considerable time (7) but only in recent years this property of EBV has been successfully exploited for the generation of human monoclonal antibodies able to neutralise the influenza virus

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(8) or block transmission of the virus (HIV) causing acquired immune deficiency syndrome (9).

A final and powerful strategy for the generation of human monoclonal entibodies for therapy involves expression of human antibody fragments on the tip of certain bacteriophages, such as the fd phage. The fd phage slows down the growth - but does not kill - infected bacterial cells (resulting in bacterial plaques) and in 1985 George Smith demonstrated that the fd phage retained infectivity even when short peptides or protein domains were fused in the sequence of the phage protein pIII, responsible for docking and enabling phage entrance (10). On the strength of this discovery, John McCafferty, Greg Winter and colleagues a few years later succeeded in selecting antibody specificities displayed on phage (11) thus paving the way for the use of this prokariotyc technology for selection of antibody specificities useful for antibody therapy. The sequences encoding these human antibody fragments can then be fused to those encoding the constant (C) domains for expression of intact immunoglobulins in suitable mammalian cell lines.

## Monoclonal antibodies in therapy

The therapeutic potential of monoclonal antibodies is vaste and will not be exhausted in this lecture. It spans applications in infectious diseased (references to influenza and AIDS have been given above), chronic inflammatory pathologies such as rheumatoid arthritis and Crohn's disease, several autoimmune pathologies including multiple sclerosis and systemic lupus erhytematosus. Remarkably there is considerable evidence demonstrating that monoclonal antibodies directed to certain antigens on cancer cells or on cytotoxic T cells may display considerable potential in the therapy of several types of human cance.

A role for the immune system in cancer surveillance was first hypotesised by Paul Ehrlich in 1909 (12) and was extensively argued and re-established half a century later by Franck M Burnet (13). In the 1970s the Ehrlich and Burnet hypothesis was tested but several studies failed to demonstrate the cancer surveillance concept with the experimental models available at the time (14). The immune surveillance of cancer growth has now been established beyond reasonable doubt (15) and a series of elegant studies have now demonstrated that the tumours that grow - in animals and most probably in man - are the ones 'selected' by the immune system, namely they are antigenically-weak tumours that escape killing by the cells of the immune system (16). These concepts have major implications for the development of novel approaches for cancer immunotherapy.

In one such strategy, molecules (so-called immune checkpoints) responsible for containing and restriciting the activity of cytotoxic T cells have been targeted with monoclonal antibodies in order to unleash the killing potential of these effector cells against 'antigenically weak' tumours. These antibodies have demonstrated therapeutic activity in patients with melanoma and carcinomas of the lung and colon (17-19) and a large number of clinical studies are now in progress to assess activity of the antibodies targeting immune checkpoints in other types of cancer. In parallel, other studies are directed at developing antibodies against surface compo-

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nents of cancer cells that can be targeted directly with therapeutic antibodies. Notable examples of this area of study are antibodies such as rituximab (anti-CD20) and trastuzumab (anti-HER2) employed in the treament of certain types of lymphomas and breast cancers.

## Conclusions

The path of therapeutic antibodies from anti-toxin to magic bullets has been a long one but is now coming full circle and it is now clear that antibodies are a major class of therapeutics for a number of human diseases, infectious and not. This result is the remarkable point of convergence of seemingly unrelated lines of work in somatic cell genetics, phage genetics, protein engineering,

X-ray crystallography, etc that - together - have yielded robust, cross-discipline technological platforms for design, selection and expression of therapeutic antibodies including human ones.

It should also be noticed that all major breakthroughs in this field, ie the somatic cell hybridisation experiments of C Milstein and G Kohler, the early phage display experiments of G Smith, P Berg's studies on viral gene sequences that later proved essential for successful expression of antibodies in mammalian cells, were the outcome of research programmes of a fundamental nature and whose scope was unrelated to the actual outcome. It is to be hoped that people and agencies in charge of funding 'translational' biomedical research will bear this point clearly in mind.

## Bibliografia

1. von Behring E, Kitasato S. Ueber das Zustandekommen der Diphtherie-Immunitat und der Tetanus-Immunitat bei thieren. Dtsch. Med. Wochenschr. 1890; 16: 1113-1114.
2. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975; 256: 495-497.
3. Neuberger MS, Williams GT, & Fox RO. Recombinant antibodies possessing novel effector functions. Nature. 1984; 312: 604-608.
4. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature. 1986; 321: 522-525.
5. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. Nat Rev Drug Discov. 2010; 9: 767-774.
6. Green LL. Transgenic mouse strains as platforms for the successful discovery and development of human therapeutic monoclonal antibodies. Curr Drug Discov Technol. 2014; 11: 74-84.
7. Miller G. Immortalization of human lymphocytes by Epstein-Barr virus. Yale J Biol Med. 1982; 55: 305-310.
8. Corti D, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science. 2011; 333: 850-856.

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9. Shingai M, et al. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. Nature. 2013; 503: 277-280.
10. Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science. 1985; 228: 1315-1317.
11. McCafferty J, Griffiths AD, Winter G, Chiswell DJ. Phage antibodies: filamentous phage displaying antibody variable domains. Nature. 1990; 348; 552-554.
12. Ehrlich P. Ueber den jetzigen stand der karzinomforschung. Ned Tijdschr Geneeskd. 1909; 5: 273-290.
13. Burnet M. Cancer - A biological approach. Br Med. 1957; 1: 779-786, 841-777.
14. Stutman O. Tumor development after 3-methylcholanthrene in immunologically deficient athymic-nude mice. Science. 1974; 183 534-536.
15. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011; 29: 235-271.
16 Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011; 331: 1565-1570.
17 Hodi FS, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363: 711-723.
18 Brahmer JR, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012; 366: 2455-2465.
19 Hamid O, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med. 2013; 369: 134-144.

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# 2

# Cancer Immunotherapy with genetically engineered T lymphocytes

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Adoptive T cell therapy is an innovative therapeutic approach, that relies on the ability of T lymphocytes to recognize and destroy specific targets on microbes and tumors through their T cell receptors (TCR). The priming of a naïve T cells, namely the first encounter of a naïve T cells with the target antigen in inflammatory conditions, leads to T cell activation and differentiation in an effector T cell, highly efficient in killing antigen bearing targets, and in a memory T cell, able to persist and provide long-term protection against diseases. Adoptive T cell therapy exploits these 2 major characteristics of T lymphocytes for cancer treatment. To be effective adoptively transferred T cells:

1. Specific for cancer antigens;
2. Able to expand and persist long-term;
3. Able to counteract the immunosuppressive signals mediated by cancer cells and by the tumor microenvironment.

Gene transfer and genome editing technologies allow to generate such potent anti-tumor living drugs. The transfer of genes encoding for chimeric antigen receptors (CAR) has clearly shown high efficacy in selected diseases. However, CAR-T cells target only antigens expressed on the surface of cancer cells. On the contrary, TCRs recognize antigen-derived peptides processed and presented on HLA molecules, thus allowing to largely increase the array of potential targets. The simple transfer of tumor specific TCR genes into T cells is affected by other limitations: genetically modified T cells shall express four different TCR chains, that might mispair, leading to unpredictable toxicity and to an overall dilution of the tumor specific TCR on lymphocyte surface, thus limiting the efficacy of the therapeutic cellular products. To overcome these issues, we developed the TCR gene editing protocol, based on the genetic disruption of the endogenous TCR genes (3, 4) followed by lentiviral mediated transfer of a tumor-specific TCR. TCR gene edited lymphocytes, proved safer and more effective than conventional TCR gene transferred cells in vitro and in animal models of acute myeloid leukemia and multiple myeloma. Early differentiated T cells, such as memory stem T cells and central memory lymphocytes, cells endowed with long term persistence capacity, can be engineered by TCR gene editing, thus allowing to produce long-lasting living drugs, with the ultimate aim of eliminating cancer cells and patrol the organism for tumor recurrence. Challenges and opportunities of genome editing of memory T cells will be discussed.



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## Bibliografia

1. Chabannon C, Kuball J, Bondanza A, Dazzi F, Pedrazzoli P, Toubert A, Ruggeri A, Fleischhauer K, Bonini C. Hematopoietic stem cell transplantation in its 60s: A platform for cellular therapies. Sci Transl Med. 2018; 10: pii: eaap9630.
2. Lim WA, June CH. The Principles of Engineering Immune Cells to Treat Cancer. Cell. 2017; 168: 724-740.
3. Provasi E, Genovese P, Lombardo A, Magnani Z, Liu PQ, Reik A, Chu V, Paschon DE, Zhang L, Kuball J, Camisa B, Bondanza A, Casorati G, Ponzoni M, Ciceri F, Bordignon C, Greenberg PD, Holmes MC, Gregory PD, Naldini L, Bonini C. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat Med. 2012; 18: 807-815.
4. Mastaglio S, Genovese P, Magnani Z, Ruggiero E, Landoni E, Camisa B, Schiroli G, Provasi E, Lombardo A, Reik A, Cieri N, Rocchi M, Oliveira G, Escobar G, Casucci M, Gentner B, Spinelli A, Mondino A, Bondanza A, Vago L, Ponzoni M, Ciceri F, Holmes MC, Naldini L, Bonini C. NY-ESO-1 TCR single edited stem and central memory T cells to treat multiple myeloma without graft-versus-host disease. Blood. 2017; 130: 606-618.
5. Cieri N, Camisa B, Cocchiarella F, Forcato M, Oliveira G, Provasi E, Bondanza A, Bordignon C, Peccatori J, Ciceri F, Lupo-Stanghellini MT, Mavilio F, Mondino A, Bicciato S, Recchia A, Bonini C. IL-7 and IL-15 instruct the generation of human memory stem T cells from naive precursors. Blood. 2013; 121: 573-584.

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# 3

# T cell therapy of infections in the immunocompromised individual

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Dramatic progress in the outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) from alternative sources, including mismatched unrelated donors, umbilical cord blood, and haploidentical related donors, has been registered over the past decade in pediatric patients, providing a chance to cure the children and adolescents with hematologic disorders in need of a transplant but lacking a compatible donor (1, 2).

After transplantation, recovery of donor-derived T cells facilitates engraftment, protection from opportunistic infections, and, in patients with malignancies, from relapse of the underlying disease. However, T cells transferred with the stem cell graft may also recognize foreign histocompatibility antigens on patient tissues and induce the graft-versus-host disease (GVHD), a significant cause of morbidity and mortality after allo-HSCT (3, 4). The continuous development of graft engineering and pharmacologic GVHD prevention strategies, together with better supportive care and optimal conditioning regimens, have significantly improved the outcomes of allo-HSCT from alternative sources (2, 5). In particular, transplantation from a full HLA-haplotype mismatched family member (haplo-HSCT), in addition to ensuring a donor for the large majority of patients, offers several other advantages, including prompt availability of the stem cell source, the possibility to select the best donor from a pool of family candidates, and immediate access to donor-derived cellular therapies either for the prevention of relapse or the treatment of infections after HSCT (6).

Despite encouraging results, viral infections are still important causes of morbidity and mortality in immunosuppressed patients following HSCT (7). This principally reflects the inability of the depressed host immune system to limit viral replication and dissemination, and loss of T cell function is central to this effect (8, 9). Despite advances in prophylactic and preemptive pharmacotherapy, antiviral therapeutics are limited by toxicity and to some extent by lack of efficacy in breakthrough infections (10). T-cell reconstitution is a key requirement for effective antiviral control following HSCT, and factors that influence the speed of T-cell recovery also impact the risk of viral infection in this period (9).

Immunotherapeutic strategies to accelerate reconstitution of virus-specific immunity and to hasten T cell recovery after HSCT remain a compelling alternative to drug treatments (11-13).



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The use of donor lymphocyte infusions (DLI) derived from seropositive stem cell donors is an effective salvage therapy for viral infections in HSCT recipients prior to T-cell recovery, but the risk of potentially severe acute or chronic graft-versus-host disease (GVHD) remained a concern (14), and prompted manipulation of donor lymphocytes to reduce alloreactivity while maintaining pathogen immune surveillance.

Two strategies have been explored to reduce the risks derived from alloreactivity associated with DLI. The first approach was based on transduction of nonspecific T cells with a retroviral construct containing suicide genes, to induce susceptibility to drug-mediated lysis in case of development of alloreactive response (15, 16). Although transfer of suicide genes have provided a safety switch to T cells, initial triggering of GVHD may still be a problem. Therefore, reconstitution of virus-specific immunity by transfer of donor-derived virus-specific T cells (VSTs), that should contain lower number of alloreactive T cells compared to DLI, is an appealing strategy to rapidly restore virus-specific immunity to prevent or treat viral diseases in this setting (12).

Early proof of principle studies demonstrated that the administration of donor-derived T cells specific for cytomegalovirus (CMV) or Epstein-Barr virus (EBV) could effectively restore virus-specific immunity and control viral infections (17, 18). Subsequent studies using different expansion or direct selection techniques have shown that donor-derived VSTs administered prophylactically or preemptively on the basis of viral DNA monitoring, confer protection in vivo after adoptive transfer in >70% of recipients (19-25).

Most of these studies have been conducted in adult recipients of T cell replete or deplete unrelated HSCT. However, the field of allo-HSCT is going towards an increased use of HLA-haploidentical family donors. Especially in the pediatric population, T-cell depleted haplo-HSCT has shown encouraging results, with the low transplant-related mortality observed almost exclusively attributable to infectious complications. Therefore, it would be important to demonstrate the feasibility of preventing viral reactivations through early administration of VSTs in the haplo-HSCT setting. So far, most of the experience is on treatment of herpesvirus infections (EBV, CMV) (22,26). However, it has been recently demonstrated that patients with multiple infections have a worse outcome (27), and in the pediatric population, the impact of other viral infections, such as adenovirus, has important implications for overall survival (28). Thus, the possibility to produce in a single process VSTs specific for multiple viruses is crucial for progress in the field. Proof of principle studies have been conducted, that demonstrated feasibility and preliminary efficacy of controlling viral reactivation after allo-HSCT by multivirus-specific VSTs of donor origin (29, 30).

The studies conducted in HSCT paved the way for similar experiences in the setting of solid organ transplantation and on patients with non-iatrogenic immune deficiencies. Our group has pioneered the use of EBV-specific VST to prevent or treat post-transplant lymphoproliferative disease after solid organ transplantation (31, 32), and is now applying this strategy also to resistant CMV disease. Likewise, we have first described the feasibility to treat polyomavirus-related progressive

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multifocal leukoencephalopathy in patients with immune deficiencies with autologous or allogeneic VSTs (25).

The challenge is now to increase availability of these T cell therapies, that have been so far employed only in few academic centers. One strategy is to select VST from a leukapheretic product after short peptide stimulation and capture of activated, IFNγ-positive cells through magnetic selection by a commercial automated platform (24). This strategy is feasible in the HSCT setting, but is generally not applicable in virus-seronegative SOT recipients or in other patients with immune deficiencies. In the latter cases, an alternative approach is to employ banked allogeneic VSTs expanded from third-party, healthy seropositive donors, selected on the basis of the best HLA match (12, 33).

## Bibliografia


1. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006; 354: 1813-1826.
2. Zecca M, Comoli P. Haploidentic SCT Applications in pediatric patients. In: T. Demirer, ed. Progress in Stem Cell Transplantation. In-Tech pub. ISBN. 978-953-51-2227-2¸ Doi: 10.5772/59336
3. Shlomchik WD. Graft-versus-host disease. Nat Rev Immunol. 2007; 7: 340-352.
4. Ferrara JLM, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009; 373: 1550-1561.
5. Locatelli F, Bernardo ME, Bertaina A, Rognoni C, Comoli P, Rovelli A, et al. Efficacy of two different doses of rabbit anti-T-lymphocyte globulin to prevent graft-versus-host disease in children with haematological malignancies transplanted from an unrelated donor: a multicentre, randomised, open-label, phase 3 trial. Lancet Oncology. 2017; 18: 1126.
6. Bertaina A, Zecca M, Buldini B, et al. Unrelated donor vs HLA-haploidentical α/β T-cell- and B-cell-depleted HSCT in children with acute leukemia. Blood. 2018; 132: 2594-2607.
7. Boeckh M, Erard V, Zerr D, Englund J. Emerging viral infections after hematopoietic cell transplantation. Pediatr Transplant. 2005; 9: 48-54.
8. Moss P, Rickinson A. Cellular immunotherapy for viral infection after HSC transplantation. Nat Rev Immunol. 2005; 5: 9-20.
9. Leen AM, Heslop HE, Brenner MK. Antiviral T-cell therapy. Immunol Rev. 2014; 258: 12-29.
10. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009; 15: 1143-238.
11. Roddie C, Peggs KS. Immunotherapy for transplantation-associated viral infections. J Clin Invest. 2017; 127: 2513-22.
12. Bollard CM, Heslop HE. T cells for viral infections after allogeneic hematopoietic stem cell transplant. Blood. 2016; 127: 3331-3340.
13. Comoli P, Zecca M, Maccario R. Immunotherapy against EBV-lymphoma in recipients of HSCT. Expert Rev Hematol. 2010; 3: 625-632.


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14. Papadopoulos EB, Ladanyi M, Emanuel D, et al. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. N Engl J Med. 1994; 330: 1185-1191.
15. Bonini C, Ferrari G, Verzeletti S, Servida P, Zappone E, Ruggieri L, et al. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. Science 1997; 276: 1719-24.
16. Kapoor N, Bertaina A, Merli P, et al. Outcome of Children with Primary Immune-Deficiencies (PIDs) enrolled in a phase I-II trial based on the infusion of BPX-501 donor t cells genetically modified with a novel suicide gene (inducible Caspase 9, iC9) after T-cell depleted HLA-haploidentical allogeneic stem cell transplantation (haplo-HSCT). Blood. 2016; 128: 4683.
17. Walter EA, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med. 1995; 333: 1038-1044.
18. Rooney CM, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-Virus-related lymphoproliferation. Lancet. 1995; 345: 9-12.
19. Einsele H, et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. Blood. 2002; 99: 3916-3922.
20. Peggs KS, et al. Adoptive cellular therapy for cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines. Lancet. 2003; 362: 1375-1377.
21. Cobbold M, Khan N, Pourgheysari B, et al. Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. J Exp Med 2005; 202: 379-386.
22. Comoli P, S Basso, M Zecca, et al. Preemptive therapy of EBV-related lymphoproliferative disease after pediatric haploidentical stem cell transplantation. Am J Transplant. 2007; 7: 1648-1655.
23. Feuchtinger T, et al. Safe adoptive transfer of virus-specific T-cell immunity for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation. Br J Haematol. 2006; 134: 64-76
24. Feuchtinger T, Opherk K, Bethge WA, et al. Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. Blood. 2010; 116: 4360.
25. Balduzzi A, Lucchini G, Hirsch HH, et al. Polyomavirus JC-targeted T-cell therapy for progressive multiple leukoencephalopathy in a hematopoietic cell transplantation recipient. Bone Marrow Transplant. 2011; 46: 987-92.
26. Pei X, et al. Cytomegalovirus-Specific T-Cell Transfer for Refractory Cytomegalovirus Infection After Haploidentical Stem Cell Transplantation: The Quantitative and Qualitative Immune Recovery for Cytomegalovirus. J Infect Dis. 2017; 216: 945-956.
27. Hill JA, Mayer BT, Xie H, et al. The cumulative burden of double-stranded DNA virus detection after allogeneic HCT is associated with increased mortality. Blood. 2017; 129: 2316-2325.

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28. Walls T, Shankar AG, Shingadia D. Adenovirus: an increasingly important pathogen in paediatric bone marrow transplant patients. Lancet Infect Dis. 2003; 3: 79-86.
29. Leen AM, et al. Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. Nature Med. 2006; 12: 1160-1166.
30. Papadopoulou E, Gerdemann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. Sci Transl Med. 2014; 6: 242ra83.
31. Comoli P, M Labirio, S Basso, et al. Infusion of autologous EBV-specific cytotoxic T cells for prevention of EBV-related lymphoproliferative disorder in solid organ transplant recipients with evidence of active virus replication Blood. 2002; 99: 2592-2598.
32. Comoli P, Maccario R, Locatelli F, et al. Treatment of EBV-related post-renal transplant lymphoproliferative disease with a tailored regimen including EBV-specific T cells. Am J Transplant. 2005; 5: 1415-1422.
33. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood. 2007; 110: 1123-1131.

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# 4

# Cell Therapy: where the injected cells go and how they change their transcriptional asset

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Mechanisms of hematopoietic reconstitution after bone marrow (BM) transplantation remain largely unknown. Current models of adult hematopoietic function consider that active bone marrow (ABM) is homogeneously distributed within the intra-osseous space of the whole axial skeleton, as well as in the hips and in the proximal epiphyses of humeri and femurs. Accordingly, regardless of the sampling site, cellular and molecular analyses based on bone marrow (BM) aspiration or biopsy represent the standard for diagnosis, staging, and response assessment in the vast majority of blood disorders. This notion implies that any modification of BM composition occurs almost synchronously in every BM district and that the circulation of a limited number of hematopoietic stem cells (HSCs) accounts for the tight adjustment of hematopoiesis to blood cell demand.

This physiological feature is a key mechanism of BM transplantation. Reconstitution of recipient hematopoietic function is possible with as little as 1% of donor BM, indicating a significant redundancy in the HSC reservoir in normal humans. This small quota of donor BM can spread throughout the different skeletal segments and, following a considerable expansion and proliferation process, restore the host cell production and maintain the hematopoietic function indefinitely.

Nevertheless, our limited knowledge about extension, distribution, and activity of transplanted BM cells in humans has prevented so far a full comprehension of determinants of its engraftment in various BM districts and its impact on transplantation outcomes. The concept of even distribution of hematopoietic cell subsets has been recently challenged in the mouse model of BM transplant, indicating that HSCs are not homogeneously distributed in the various BM areas. In addition, that study showed that granulocyte colony-stimulating factor (G-CSF) treatment plays a role in redistributing HSCs. On the other hand, the murine hematopoietic system significantly differs from human BM, as mice cannot possibly expand their hematopoietic system because their baseline blood elements production effort employs the whole BM as well as the spleen. Conversely, in adult humans, only a proportion of BM spaces are occupied by functioning marrow.

Some simple mathematical considerations might help to better define the importance of volumetric availability in the context of HSC turnover: overall, BM produces an average of 10^11 granulocytes and 10^11 erythrocytes per day. Because volumes of these cells are 200 to 300 fL and 90 fL, respectively, this activity



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roughly corresponds to a BM cell output averaging 30 to 50 mL per day. Considering a total BM asset of 10^12 cells corresponding to 520 mL, this would imply a cell renewal rate of 10% per day, to account for the baseline physiological regenerative demand of adult subjects.

In the post-transplant setting, engrafted HSCs are required to undergo a tremendous proliferative effort to restore the normal blood elements values in the shortest possible time. The reconstitution ad integrum of the entire stem cell pool remains disputed, but this is beyond the scope and the possibilities of the present approach. Restoration of normal hematopoiesis could be theoretically met using different strategies. In the first scenario, HSCs could increase their proliferation rate within the standard active hematopoietic sites. Alternatively, HSCs could recolonize intraosseous spaces that were abandoned because they were redundant for the standard homeostatic need, thus restoring the hematopoietic asset classically described in infancy.

These two different patterns would imply divergent signaling mechanisms, whose relative contribution is yet undefined, because of the obvious concerns in performing repeated BM biopsies in multiple bone segments. Limited data are available on BM extension, distribution, and activity following HSC transplant, thus limiting our comprehension of the pathophysiological aspect of HSC engraftment and the impact of these parameters on the subsequent outcome. Such a limitation can be at least partially overcome by imaging approaches with positron emission tomography (PET)/computed tomography (CT) whose computational analysis has been shown to provide an accurate assessment of BM extension, distribution, and metabolic activity.

We applied this validated software tool to a series of patients evaluated after IV adult BM transplantation (allogeneic cell transplantation [ACT]) or intrabone cord blood trans- plantation (CBT) to verify the homing features of transplanted HSCs.

We applied a computational quantification software application to hybrid 18F-fluorodeoxyglucose positron emission tomography (PET)/computed tomography (CT) images to assess activity and distribution of the hematopoietic system throughout the whole skeleton of recently transplanted patients. Thirty-four patients underwent PET/CT 30 days after either adult stem cell transplantation (allogeneic cell transplantation [ACT] or cord blood transplantation (CBT). Our software automatically recognized compact bone volume and trabecular bone volume (IBV) in CT slices. Within IBV, co-registered PET data were extracted to identify the active BM (ABM) from the inactive tissue. Patients were compared with 34 matched controls chosen among a published normalcy database. Whole body ABM increased in ACT and CBT when compared with controls (12.4 6 3 and 12.8 6 6.8 vs 8.1 6 2.6 mL/kg of ideal body weight [IBW], $P < .001$). In long bones, ABM increased three- and sixfold in CBT and ACT, respectively, compared with controls (0.9 6 0.9 and 1.7 6 2.5 vs 0.3 6 0.3 mL/kg IBW, $P < .01$). These data document an unexpected distribution of transplanted BM into previously abandoned BM sites.

As already stated above, it is generally assumed that total BM cellularity of approximately 10^12 cells; thus, can be extrapolated that CD34+ cells expand approximately by a factor of 2 logs after bone marrow transplant (BMT) and 3 logs

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after umbilical cord blood transplant (UCBT). The magnitude of expansion is probably higher since we should take into account the impact of seeding efficiency. It has been argued since long time that HSC might undergo some sort of exhaustion after transplantation. This concept mainly derives from studies of serial transplantation in mice and of transplants in humans where the frequency of long-term culture-initiating cells (LTC-IC) has been shown to be permanently reduced after transplantation. However, Iscove and Nawa have elegantly disputed this concept. Curiously, it was shown that in children after UCBT the reconstitution of the HSC reservoir (operationally LTC-IC) was superior compared to that of children given adult HSCs (i.e., BM cells), notwithstanding both neutrophil and platelet recovery is delayed. Thus, CB HSCs seem to display very efficient self-renewal machinery.

To investigate how HSC reorganize their transcriptional asset to cope with the need of hematopoietic regeneration, we evaluated the expression of 91 genes selected for their role in self- renewal and maintenance of stemness. We have evaluated the transcriptional asset in CD34+cells obtained from baseline BM or UCB units and from patients transplanted with either adult or UCB cells. Thus, we investigated the self-renewal program of HSC of different origins by making use of different donor/recipient combinations. First, an exploratory analysis was performed to disclose a set of genes significantly up-regulated in transplanted CD34+cells. Then, a multivariate sparsity-inducing machine learning algorithm was used to identify four gene signatures with remarkably accurate predictive capabilities. Furthermore, the four signatures underwent a functional characterization procedure that lead to a set of meaningful KEGG pathways as well as an inferred network of gene associations.

We investigated the gene expression of HSC (operationally defined as CD34+ cells) from adult bone marrow (ABM) and from Cord Blood (CB) in steady state and after transplantation. We concentrated our attention to a relatively restricted number of genes by choosing those considered relevant in in self-renewal and expansion of HSC according to the data of the literature. Specifically we evaluated the expression of ninety-one  genes that were analyzed by Real-Time PCR in CD34+ cells isolated from samples derived from four different sources:

1) 12 samples from Umbilical Cord Blood (UCB);
2) 15 samples from Bone Marrow healthy donors;
3) 13 samples from Bone Marrow after Umbilical Cord Blood Transplant (UCBT);
4) 29 patients from Bone Marrow after transplantation with adult HSC.

First, univariate analyses, using Mann-Whitney test, were performed to disclose sets of genes significantly up or down regulated; subsequently multivariate machine learning analyses (MMLA), using an implementation of the elastic net algorithm, were performed. The MMLA showed that each of the four types of CD34+ cells overexpress a well defined set of genes. This allows identifying four specific gene signatures. Namely, by analyzing a specific signature one can identify a CD34+cell source with 80-90% of likelihood. The reliability of these results is guaranteed by the two nest K-fold cross-validation loops where the model selection and the classification accuracy are assessed.

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Interestingly, in the comparison of UCBT vs HSCT we found that CD34+ cells after UCBT displayed a signature remarkably divergent compared to CD34+ cells after transplantation of adult HSC, suggesting that HSC from different sources utilize different program to expand and repopulate the hematopoietic system. Curiously we found, an overexpression in CD34+ cells after UCBT of a set of genes playing a key role in reprogramming somatic cells, namely DPPA2, LIN28, NANOG, NES, OCT4, SOX1, SOX2 and PTEN genes; this was not observed after adult transplant.

Unexpectedly, the gene signature of adult HSC changes after transplantation and remains different from the donor signature after months or years. We have no evidence that HSC return to the original gene signature after transplantation.

Despite the robustness and reliability of the results, our study is still limited by the number of collected samples;

In conclusion, transplant imposes a new transcriptional asset in CD34+ cells that differs according to the origin of HSC. MMLA allows disclosing that transplanted CD34+ cells from adult cells acquire an asset very different from transplanted CD34+ cells from cord blood. In many cases transplanted HSC from CB overexpress reprogramming genes. Grafted HSC change their gene expression profile without returning to the original pre-transplant asset even when a steady state has been reached. However, this big change does not alter the commitment to hematopoietic lineage. Overall, these results reveal undisclosed aspects of transplantation biology.

**Bibliografia**

1. Hayman JA, Callahan JW, Herschtal A, et al. Distribution of proliferating bone marrow in adult cancer patients determined using FLT-PET imaging. Int J Radiat Oncol Biol Phys. 2011; 79: 847-852.
2. Bhojwani D, Howard SC, Pui CH. High-risk childhood acute lymphoblastic leukemia. Clin Lymphoma Myeloma. 2009; 9 (suppl. 3): S222-S230.
3. Uchida N, Aguila HL, Fleming WH, Jerabek L, Weissman IL. Rapid and sustained hematopoietic recovery in lethally irradiated mice transplanted with purified Thy-1.1lo Lin-Sca-11 hematopoietic stem cells. Blood. 1994; 83: 3758-3779.
4. Verovskaya E, Broekhuis MJ, Zwart E, et al. Asymmetry in skeletal distribution of mouse hematopoietic stem cell clones and their equilibration by mobilizing cytokines. J Exp Med. 2014; 211: 487-497.
5. Shehee WR, Oliver P, Smithies O. Lethal thalassemia after insertional disruption of the mouse major adult beta-globin gene. Proc Natl Acad Sci USA. 1993; 90: 3177-3181.
6. Koury MJ. Abnormal erythropoiesis and the pathophysiology of chronic anemia. Blood Rev. 2014; 28: 49-66.
7. Ting-Beall HP, Needham D, Hochmuth RM. Volume and osmotic properties of human neutrophils. Blood. 1993; 81: 2774-2780.
8. McLaren CE, Brittenham GM, Hasselblad V. Statistical and graphical evaluation of erythrocyte volume distributions. Am J Physiol. 1987; 252: H857-H866.

TORNA ALL'INDICE

9. Podesta M, Piaggio G, Frassoni F, et al. The assessment of the hematopoietic reservoir after immunosuppressive therapy or bone marrow transplantation in severe aplastic anemia. Blood. 1998; 91: 1959-1965.
10. Sambuceti G, Brignone M, Marini C, et al. Estimating the whole bone-marrow asset in humans by a computational approach to integrated PET/CT imaging. Eur J Nucl Med Mol Imaging. 2012; 39: 1326-1338.
11. Iscove NN, Nawa K. Hematopoietic stem cells expand during serial transplantation in vivo without apparent exhaustion. Curr Biol. 1997; 7: 805-808.
12. Aoki J, Ohashi K, Mitsuhashi M, et al. Posttransplantation bone marrow assessment by quantifying hematopoietic cell-derived mRNAs in plasma exosomes/microvesicles. Clin Chem. 2014; 60: 675-682.
13. Cristy M. Active bone marrow distribution as a function of age in humans. Phys Med Biol. 1981; 26: 389-400.
14. Yao WJ, Hoh CK, Hawkins RA, et al. Quantitative PET imaging of bone marrow glucose metabolic response to hematopoietic cytokines. J Nucl Med. 1995; 36: 794-799.
15. Knopp MV, Bischoff H, Rimac A, Oberdorfer F, van Kaick G. Bone marrow uptake of fluorine-18- fluorodeoxyglucose following treatment with hematopoietic growth factors: initial evaluation. Nucl Med Biol. 1996; 23: 845-849.
16. Sugawara Y, Fisher SJ, Zasadny KR, Kison PV, Baker LH, Wahl RL. Preclinical and clinical studies of bone marrow uptake of fluorine-1- fluorodeoxyglucose with or without granulocyte colony-stimulating factor during chemotherapy. J Clin Oncol. 1998; 16: 173-180.
17. Murata Y, Kubota K, Yukihiro M, Ito K, Watanabe H, Shibuya H. Correlations between 18F-FDG uptake by bone marrow and hematological parameters: measurements by PET/CT. Nucl Med Biol. 2006; 33: 999-1004.
18. Frassoni F, Gualandi F, Podesta` M, et al. Direct intrabone transplant of unrelated cord-blood cells in acute leukaemia: a phase I/II study. Lancet Oncol. 2008; 9: 831-839.
19. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006; 354: 1813-1826.
20. Robinson JD, Lupkiewicz SM, Palenik L, Lopez LM, Ariet M. Determination of ideal body weight for drug dosage calculations. Am J Hosp Pharm. 1983; 40: 1016-1019.
21. Avivi I, Zilberlicht A, Dann EJ, et al. Strikingly high false positivity of surveillance FDG-PET/CT scanning among patients with diffuse large cell lymphoma in the rituximab era. Am J Hematol. 2013; 88: 400-405.
22. Tang B, Patel MM, Wong RH, et al. Revisiting the marrow metabolic changes after chemotherapy in lymphoma: a step towards personalized care. Int J Mol Imaging. 2011; 2011: 942063.
23. Ulaner GA, Lyall A. Identifying and distinguishing treatment effects and complications from malignancy at FDG PET/CT. Radiographics. 2013; 33: 1817-1834.
24. Rose BS, Liang Y, Lau SK, et al. Correlation between radiation dose to 18F-FDG-PET defined active bone marrow subregions and acute hematologic

TORNA ALL'INDICE

toxicity in cervical cancer patients treated with chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2012; 83: 1185-1191.
25. Park S, Lee SJ, Chang WJ, et al. Positive correlation between baseline PET or PET/CT findings and clinical parameters in multiple myeloma patients. Acta Haematol. 2014; 131: 193-199.
26. Frassoni F, Podesta M, Maccario R, et al. Cord blood transplantation provides better reconstitution of hematopoietic reservoir compared with bone marrow transplantation. Blood. 2003; 102: 1138-1141.
27. Frisch BJ, Ashton JM, Xing L, Becker MW, Jordan CT, Calvi LM. Functional inhibition of osteoblastic cells in an in vivo mouse model of myeloid leukemia. Blood. 2012; 119: 540-550.
28. Secchiero P, Corallini F, Barbarotto E, et al. Role of the RANKL/RANK system in the induction of interleukin-8 (IL-8) in B chronic lymphocytic leukemia (B-CLL) cells. J Cell Physiol. 2006; 207: 158-164.
29. Fiz F, Marini C, Piva R, et al. Adult advanced chronic lymphocytic leukemia: computational analysis of whole-body CT documents a bone structure alteration. Radiology. 2014; 271: 805-813.
30. Basu S, Houseni M, Bural G, et al. Magnetic resonance imaging based bone marrow segmentation for quantitative calculation of pure red marrow metabolism using 2-deoxy-2-[F-18] fluoro-D-glucose-positron emission tomography: a novel application with significant implications for combined structure-function approach. Mol Imaging Biol. 2007; 9: 361-365.
31. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic- cell transplantation. The New England journal of medicine. 2010; 363: 2091-2101.
32. van Hennik PB, de Koning AE and Ploemacher RE. Seeding efficiency of primitive human hematopoietic cells in nonobese diabetic/severe combined immune deficiency mice: implications for stem cell frequency assessment. Blood. 1999; 94: 3055-3061.
33. Harrison DE and Astle CM. Loss of stem cell repopulating ability upon transplantation. Effects of donor age, cell number, and transplantation procedure. The Journal of experimental medicine. 1982; 156: 1767-1779.
34. Podesta M, Piaggio G, Frassoni F, et al. The assessment of the hematopoietic reservoir after immunosuppressive therapy or bone marrow transplantation in severe aplastic anemia. Blood. 1998; 91: 1959-1965.
35. Iscove NN and Nawa K. Hematopoietic stem cells expand during serial transplantation in vivo without apparent exhaustion. Current biology : CB. 1997; 7: 805-808.
36. Frassoni F, Podesta M, Maccario R, et al. Cord blood transplantation provides better reconstitution of hematopoietic reservoir compared with bone marrow transplantation. Blood. 2003; 102: 1138-1141.
37. Frassoni F, Gualandi F, Podesta M, et al. Direct intrabone transplant of unrelated cord-blood cells in acute leukaemia: a phase I/II study. The Lancet Oncology. 2008; 9: 831-839.
38. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. Nature. 2008; 451: 141-146.

TORNA ALL'INDICE

39. Warlich E, Kuehle J, Cantz T, et al. Lentiviral vector design and imaging approaches to visualize the early stages of cellular reprogramming. Molecular therapy ì: the journal of the American Society of Gene Therapy. 2011; 19: 782-789.
40. Aasen T, Raya A, Barrero MJ, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nature biotechnology. 2008; 26: 1276-1284.
41. Cilloni D, Carturan S, Bracco E, et al. Aberrant activation of ROS1 represents a new molecular defect in chronic myelomonocytic leukemia. Leukemia research. 2013; 37: 520-530.
42. De Mol C, Mosci S, Traskine M and Verri A. A regularized method for selecting nested groups of relevant genes from microarray data. Journal of computational biology: a journal of computational molecular cell biology. 2009; 16: 677-690.
43. De Mol C DVE, Rosasco L. Elastic net regularization in learning theory. Journal of Complexity. 2009. 14. Zou HaH, T. Regularization and variable selection via the elastic net. Journal of the Royal Statistical Society. 2005.
44. Fardin P, Barla A, Mosci S, Rosasco L, Verri A and Varesio L. The l1-l2 regularization framework unmasks the hypoxia signature hidden in the transcriptome of a set of heterogeneous neuroblastoma cell lines. BMC genomics. 2009; 10: 474.
45. Fardin P, Cornero A, Barla A, et al. Identification of multiple hypoxia signatures in neuroblastoma cell lines by l1-l2 regularization and data reduction. Journal of biomedicine & biotechnology. 2010; 2010: 878709.
46. Mascelli S, Barla A, Raso A, et al. Molecular fingerprinting reflects different histotypes and brain region in low grade gliomas. BMC cancer. 2013; 13: 387.
47. Squillario M and Barla A. A computational procedure for functional characterization of potential marker genes from molecular data: Alzheimer's as a case study. BMC medical genomics. 2011; 4: 55.
48. Barla A MS, Rosasco L, Verri A. A method for robust variable selection with significance assessment. Proceedings of ESANN. 2008.
49. Jurman G, Merler S, Barla A, Paoli S, Galea A and Furlanello C. Algebraic stability indicators for ranked lists in molecular profiling. Bioinformatics. 2008; 24: 258-264.
50. Fardin P, Barla A, Mosci S, et al. A biology-driven approach identifies the hypoxia gene signature as a predictor of the outcome of neuroblastoma patients. Molecular cancer. 2010; 9: 185.
51. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131: 861-872.
52. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318: 1917-1920.
53. Gratwohl A, Pasquini MC, Aljurf M, et al. One million haemopoietic stem-cell transplants: a retrospective observational study. The Lancet Haematology. 2015; 2: e91-100.
54. Thorsteinsdottir U, Sauvageau G and Humphries RK. Enhanced in vivo regenerative potential of HOXB4-transduced hematopoietic stem cells with regulation of their pool size. Blood. 1999; 94: 2605-2612.

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55. Graham V, Khudyakov J, Ellis P and Pevny L. SOX2 functions to maintain neural progenitor identity. Neuron. 2003; 39: 749-765.
56. Hochedlinger K and Jaenisch R. Induced Pluripotency and Epigenetic Reprogramming. Cold Spring Harbor perspectives in biology. 2015; 7.
57. Nichols J, Zevnik B, Anastassiadis K, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell. 1998; 95: 379-391.
58. Pan G and Thomson JA. Nanog and transcriptional networks in embryonic stem cell pluripotency. Cell research. 2007; 17: 42-49.
59. Young RA. Control of the embryonic stem cell state. Cell. 2011; 144: 940-954.
60. Giorgetti A, Montserrat N, Aasen T, et al. Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. Cell stem cell. 2009; 5: 353-357.
61. Ito K, Bernardi R and Pandolfi PP. A novel signaling network as a critical rheostat for the biology and maintenance of the normal stem cell and the cancer-initiating cell. Current opinion in genetics & development. 2009; 19: 51-59.
62. Yilmaz OH, Valdez R, Theisen BK, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature. 2006; 441: 475-482.
63. Zhang J, Grindley JC, Yin T, et al. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature. 2006; 441: 518-522.
64. Guo W, Lasky JL, Chang CJ, et al. Multi-genetic events collaboratively contribute to Pten- null leukaemia stem-cell formation. Nature. 2008; 453: 529-533.

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# 5

# Mielofibrosi idiopatica: una immunoterapia è possibile?

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Myelofibrosis (MF) is a classical Philadelphia-negative chronic myeloproliferative neoplasm due to the clonal proliferation of a hematopoietic stem/progenitor cell, of unknown etiology (1). The clinical course of the disease can be heterogeneous, being characterized from the beginning by proliferation of the erythroid, as well as myeloid and megakaryocytic lineages (resulting in polyglobulia, leukocytosis, and thrombocytosis). However, in some cases the disease onset is characterized by anemia or leukopenia or thrombocytopenia; cytopenia(s), in particular anemia, is often the final evolution of the disease, although it should be underlined that 15-20% of patients progress toward a leukemic transformation (2). The disease course is almost invariably characterized by splenomegaly, that can reach massive size, and by the occurrence of systemic symptoms such as fever, night sweats, and weight loss. Muscular and joint pain, pruritus (exacerbated by the contact with water) and a tendency to develop thrombotic events (often in the splanchnic district) that not infrequently represent the first sign of the disease, complete the clinical presentation of the disease. This clinical presentation is similar both in the case that the disease develops on its own as "primary" myelofibrosis (PMF) and in the case that myelofibrosis progresses from a pre-existing condition of Polycythemia Vera (PV; post PV-MF) or Essential Thrombocythemia (ET; post ET-MF) (3). A variable degree of bone marrow fibrosis is detectable at marrow biopsy examination; however, fibrosis is not an essential histologic requisite for the diagnosis of the disease: a pre-fibrotic condition (pre-fibrotic MF) with clinical peculiar characteristics, has been recognized by WHO (4-6). In fact, the patognomic histologic feature of the disease is the presence of an increased number of abnormal, tightly clustered, megakaryocytes in the bone marrow (4). The pathogenesis of the disease is still matter of debate: a relevant role has been recently attributed to the constitutive activation of the JAK-STAT pathway in the hematopoietic cells, due to an acquired gain of function mutations either in the JAK2 or in the MPL or in the CALR genes (7). One of these so-called "driver mutations" is detectable in about 90% of patients with PMF. Besides the activation of the JAK-STAT pathway, an immune system dysregulation with an overproduction of pro-inflammatory, pro-angiogenic and pro-fibrogenic cytokines is thought to play a role in the pathogenesis of the systemic symptoms, the neoangiogenetic and the pro-fibrotic processes that characterize

the disease (8). Treatment of MF is often based on the clinical needs of the patients (9); although in recent years new drugs (10) and new approaches (including immunotherapy, see below) have been developed, the only current curative option for the disease is the allogenic hematopoietic stem cell transplantation that can be offered to a small number of patients (11). The prognosis of PMF is variable and the median survival is estimated around at 6 years from diagnosis, ranging widely from 2 years to 15 (and in some cases even more) years (12).

Considering the pro-inflammatory status and the dysregulated immune system of MF patients (8, 13-17), it is not surprising that in recent years new immune-based therapeutic approaches have been tested. These include immunomodulatory imide drugs (IMiDs), interferons (INFs), monoclonal antibodies (MoAbs) and immune checkpoint inhibitors either alone or in various combinations with standard drugs, such as hydroxyhurea or JAK-inhibitors (18). In particular, IMiDS (thalidomide, lenalidomide and pomalidomide) have been proved to reduce anemia and thrombocytopenia, as well as, although at a lesser extent, splenomegaly in patients with PMF (19-21). Their mechanism of action in PMF is based on their anti-inflammatory, anti-proliferative, anti-angiogenic and immunomodulatory activity which stem from their ability to inhibit the transcription factor NF-kB which mediates the activity of proimflammatory cytokine such as IL2, IL6, IL8, IL10, TNFalpha, TGFbeta and VEGF. In addition, they can up-reguate INFgamma and IL2 levels, as well as display a direct activity on cytotoxic/regulatory T cells and NK cells. Based on these evidences, multiple phase I/II clinical trial based on these drugs have been carried out in recent years: despite the strong rationale for their efficacy in PMF patients, no clear agreement on the use of IMiDs in PMF is emerged from these trials. A variable degree of improvement of anemia and/or thrombocytopenia was observed in 1/3 of patients without a clear cut evidence of benefit on splenomegaly.[18] Results were not improved by the concomitant admistration of JAK-inhibitors or corticosteroids. Interferon-alpha have been successfully used for the treatment of patients with PV or ET (22). Its use in PMF is supported by its capacity to increase the expression of proapoptotic genes, to decrease cell proliferation and angiogenesis. More importantly, it has been shown that INFalpha treatment results in the reduction, and sometimes the eradication, of the JAK2V617F clone, inducing a partial/complete molecular remission (23). Nevertheless, a specific role for INFalpha for the treatment of PMF is still matter of debate, due to the limited number of randomized studies, the small sample size and a certain difficulty in maintaining the dose during the studies, because of the incidence of side effects. More recently, the development of INFalpha formulation (Pegylated-Interferon) endowed with lower toxicity profile has proposed INFalpha as a promising agent for trials in PMF, especially for young patients, alone or in combination with JAK-inhibitors (24). The use of monoclonal antibodies targeting molecules or enzymes, especially of the microenvironment, involved in the pathogenesis of PMF (lysyl oxidase, TGF-beta and VEGF) resulted in few clinical trial with limited activity sometimes associated to high toxicity (18). Recently, CD123, a receptor with high affinity for IL-3 was shown to be overexpressed in myeloproliferative diseases, including MF, as well as in other hematologic malignancies (25). Thus, an anti-CD123 fusion protein (SL-

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401) was developed to target CD123, which is currently tested in an ongoing clinical trial recruiting patients with MPNs (26). Finally, T-cell targeted therapies aimed at achieve optimal antitumor immunity has been recently developed. PD-1/PD-L1 and CTLA4 inhibitors has been used for the experimental treatment of myeloid malignancies with promising results (27). Although our knowledge on the role of immune checkpoint inhibitors in PMF is still limited, two clinical trials evaluating PD-1 inhibition in PMF patients are currently ongoing. Immune checkpoint agents could also be envisaged in combination therapy with other drugs acting on different pathogenetic pathways, thus enhancing the response taking advantage from the distict mechanism of action (18).

Immunotherapy is currently considered a breakthrough in cancer therapy, and recent successes in hematological malignancies such as Hodgkin's lymphoma, multiple myeloma, myelodysplastic syndromes, and acute myeloid leukemias has driven immunotherapy towards other hematological malignancies, including myelofibrosis (28). Given the poor outcome of patients with advanced PMF and the substantial lack of drugs and compounds that can effectively affects the biology of the disease, the availability of new classes of immune modulators and/or conjugated toxins against specific targets can represent the real step forward novel therapeutic approaches for the cure of the disease in a hopefully very close future.

## Bibliografia

1. Skoda R. The genetic basis of myeloproliferative disorders. Hematology Am Soc Hematol Educ Program. 2007; 1: 1-10.
2. Vannucchi AM, Guglielmelli P, Tefferi A. Advances in understanding and management of myeloproliferative neoplasms. CA Cancer J Clin. 2009; 59: 171-91.
3. Barbui T, Barosi G, Birgegard G, et al. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from the European LeukemiaNet. J Clin Oncol. 2011; 29: 761-770.
4. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127: 2391-2405.
5. Mudireddy M, Shah S, Lasho T, et al. Prefibrotic versus overtly fibrotic primary myelofibrosis: clinical, cytogenetic, molecular and prognostic comparisons. Br J Haematol. 2017.
6. Barosi G, Rosti V, Bonetti E, et al. Evidence that prefibrotic myelofibrosis is aligned along a clinical and biological continuum featuring primary myelofibrosis. PLoS One. 2012; 7: e35631.
7. Vainchenker W and Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017; 129: 667-679.
8. Koschmieder S, Mughal TI, Hasselbalch HC, et al. Myeloproliferative neoplasms and inflammation: whether to target the malignant clone or the inflammatory process or both. Leukemia. 2016; 30: 1018-1024.
9. Iurlo A, Cattaneo D. Treatment of myelofibrosis: old and new strategies. Clin Med Insights Blood Disord. 2017; 10: 1179545X17695233.

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10. Shreenivas A, Mascarenhas J. Emerging drugs for the treatment of myelofibrosis. Expert Opin Emerg Drugs. 2018; 23: 37-49.
11. Kröger NM, Deeg JH, Olavarria E, et al. Indication and management of allogeneic stem cell transplantation in primary myelofibrosis: a consensus process by an EBMT/ELN international working group. Leukemia. 2015; 29: 2126-2133.
12. Rumi E, Cazzola M. Diagnosis, risk stratification, and response evaluation in classical myeloproliferative neoplasms. Blood. 2017; 129: 680-692.
13. Hermouet S, Hasselbalch HC, Čokić V. Mediators of inflammation in myeloproliferative neoplasms: state of the art. Mediators Inflamm. 2015; 2015: 964613.
14. Kristinsson SY, Landgren O, Samuelsson J, Bjorkhalen M, Godin LR. Autoimmunity and the risk of myeloproliferative neoplasms. Haematologica. 2010; 95: 1216-1220.
15. Barosi G, Magrini U, Gale RP. Does autoimmunity contribute to anemia in MPN-associated myelofibrosis? Leuk Res. 2010; 34: 1119-1120.
16. Massa M, Campanelli R, Fois G, et al. Reduced frequency of circulating CD4+CD25brightCD127low FOXP3+ regulatory T cells in primary myelofibrosis. Blood. 2016; 128: 1660-1662.
17. Wang JC, Sindhu H, Chen C, et al. Immune derangements in patients with myelofibrosis: the role of Treg, Th17, and sIL-2Ralpha. PLoS One. 2015; 10: e0116723.
18. Masarova L, Verstovsek S, Kantarjian H, Daver N. Immunotherapy-based approaches in myelofibrosis. Exp Rev Hematol. 2017; 10: 903-914.
19. Marchetti M, Barosi G, Balestri F, et al. Low-dose thalidomide ameliorates cytopenias and splenomegaly in myelofibrosis with myeloid metaplasia: a phase II trial. J Clin Oncol. 2004; 22: 424-431.
20. Quintas-Cardama A, Kantarjian HN, Manshouri T, et al. Lenalidomide plus prednisone results in durable clinical, histopathological, and molecular responses in patients with myelofibrosis. J Clin Oncol. 2009: 27: 4760-4766.
21. Daver N, Shastri A, Kadia T, et al. Phase II study of pomalidomide in combination with prednisone in patients with myelofibrosis and significant anemia. Leuk Res. 2014; 38: 1126-1129.
22. Silver R, Kiladjian JJ, Hasselbalch HC. Interferon and the treatment of polycythemia vera, essential thrombocythemia, and myelofibrosis. Expert Rev Hematol. 2013; 6: 49-58.
23. Silver RT, Vandris K. Recombinant interferon alpha may retard progression of early primary myelofibrosis. Leukemia. 2009; 23: 1366-1369.
24. Gisslinger H, Zagrijtschuk O, Buxhofer-Ausch V, et al. Ropeginterferon alfa-2b, a novel IFNalpha-2b. induces high response rates with low toxicity in patients with polycythemia vera. Blood. 2015; 126: 1762-1769.
25. Lasho TFC, Kimlinger TK, Zblewski D, et al. Expression of CD123 (IL-3R-alpha), a therapeutic target of SL-401, on myeloproliferative neoplasms. Blood. 2014; 124: 5577.
26. Patnaik MGV, Gotlib JR, Carraway HE, et al. Results from ongoing phase 2 tri-

TORNA ALL'INDICE

al of SL-410 in patients with advanced, high risk myeloproliferative neoplasms including chronic myelomonocytic leukemia. Blood. 2016; 128: 4245.
27. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2009; 458: 206-210.
28. Holstrom MO and Hasselablch HC. Cancer immune therapy for myeloid malignancies: present and future. Semin Immunopathol. 2019; 41: 97-109.

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# 6

# Dal trapianto alle terapie cellulari

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Il trapianto allogenico di cellule staminali ematopoietiche è stato il primo esempio di immunoterapia cellulare e nonostante sia stato per la prima volta eseguito negli anni 60 (1), rimane a tutt'oggi l'unica procedura terapeutica in grado di garantire la guarigione di vari disordini onco-ematologici. Scopo del trapianto allogenico è la completa sostituzione dell'ematopoiesi del ricevente con quella del donatore, condizione clinica definita dal termine di "chimera completa". Questa situazione clinica viene raggiunta dopo che il tessuto ematopoietico del paziente è stato completamente eradicato dalla somministrazione di un protocollo di chemio-radioterapia sovra-massimale e la ricostituzione ematopoietica avvenuta ad opera della sospensione di cellule staminali del donatore. Quest'ultima oltre a contenere le cellule staminali del donatore contiene anche le sue cellule immunocompetenti che riconoscono come estranee (non-self) e distruggono le cellule dell'ospite (paziente). Le cellule immunocompetenti del donatore sono quindi responsabili sia di una delle maggiori complicanze del trapianto allogenico, la malattia da trapianto verso l'ospite ("graft versus host disease", GVHD), principale causa della morbilità e mortalità post-trapianto, ma anche del riconoscimento e della distruzione delle cellule leucemiche/neoplastiche sopravvissute al regime di condizionamento (Graft versus leukemia, GVL) (2, 3). È questa reazione immunologica che può determinare la completa eradicazione della malattia neoplastica residua nel paziente ad alto rischio di recidiva (4). Vari studi osservazionali hanno dimostrato che GVHD e GVL sono strettamente legate ed a tutt'oggi i numerosi tentativi finalizzati ad una loro separazione sono stati infruttuosi. Nonostante questo, i meccanismi cellulari e molecolari responsabili di GVHD e GVL sono stati progressivamente chiariti ed è oggi chiaro che i linfociti T del donatore svolgono un ruolo cruciale nel determinare l'effetto GVL. Per molti anni e prima dell'avvento degli inibitori delle tirosine kinasi il trapianto allogenico era l'unica procedura terapeutica in grado di guarire pazienti affetti da leucemia mieloide cronica (LMC) e inizialmente si riteneva che questa capacità terapeutica dipendesse da un effetto GVL innescato dai linfociti T citotossici del donatore (4, 5). Successivamente, questa suggestione aveva trovato una conferma nel fatto che l'eliminazione delle cellule T (T deplezione, TD) dalla sospensione cellulare del donatore riduceva l'incidenza di GVHD ma aumentava l'incidenza di recidiva forse perché eliminava l'interazione tra le cellule presentanti l'antigene dell'ospite e cellule T regolatorie del donatore (6). Questa stretta interazione era stata confermata da uno



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studio clinico successivo che aveva confrontato pazienti con LMC che avevano ricevuto un trapianto non-TD con quelli che avevano ricevuto trapianti TD (7). I pazienti che avevano ricevuto un trapianto TD presentavano una minor incidenza di GVHD, ma una maggior mortalità peri-trapiantologica ed una maggior incidenza di recidiva rispetto ai pazienti non TD. Tuttavia, se i pazienti TD ricevevano un'infusione di linfociti del loro donatore ("Donor Lymphocyte Infusion", DLI) la sopravvivenza diveniva uguale a quella dei pazienti TD (7). L'impiego di trapianti T depleti è avvenuto anche in altri disordini onco-ematologici ma ha sempre avuto scarso successo.

La cellula "natural killer" (NK) è un'altra cellula il cui ruolo nelle reazioni immunologiche post-trapianto è stato progressivamente e sempre meglio definito tanto che cellule NK vengono sempre più spesso impiegate in protocolli di terapie cellulari. In particolare, nei trapianti "KIR mismatched" le cellule natural killer (NK) allo-reattive del donatore riducono l'incidenza di recidiva leucemica senza far aumentare il rischio di GVHD (9-14). In questo tipo di trapianto la diversità ("mismatch") tra donatore e ricevente per il "killer cell immunoglobulin-like receptor" (KIR)" espresso dalle cellule NK media un effetto GVL attraverso il riconoscimento dell'antigene HLA di classe I non espresso dal ricevente. L'efficacia di questa azione protettiva KIR-mediata dipende dall'aplotipo HLA e dal tipo di disordine onco-ematologico del paziente. Negli ultimi anni questo effetto KIR-mediato è stato sempre più ricercato poiché l'indicazione al trapianto si è estesa ad un maggior numero di disordini onco-ematologici determinando un conseguente aumento della richiesta di donatori da registro e aplo-identici il cui impiego comporta però un più alto rischio di GVHD, un'intensificazione dei protocolli d'immunosoppressione ed un aumento del rischio infettivo. Nonostante questo, la mortalità peri-trapiantologica si è oggi progressivamente ridotta per un significativo miglioramento della terapia di supporto che utilizza terapie anti-infettive sempre più efficaci e sofisticate come ad esempio cellule T virus specifiche (15-33). Queste ultime si sono rivelate molto efficaci nella profilassi dell'infezione da Citomegalovirus, nel trattamento delle infezioni refrattarie e dei disordini linfoproliferativi secondari all'attivazione di adenovirus e del virus di Epstein-Barr. Anche la GVHD ha pure beneficiato dell'immuno-terapia cellulare visto che la capacità immuno-modulante delle cellule stromali mesenchimali (CSM) ha permesso di ottenere risposte nell'8-83% dei pazienti con GVHD acuta steroide resistente ed un piccolo trial clinico ha mostrato che le CSM riducono anche l'incidenza di GVHD cronica (34, 45).

Tutte queste conoscenze fornite dal trapianto allogenico hanno costituito il fondamento per lo sviluppo delle nuove terapie cellulari che comprendono la vaccinazione antitumorale mediante cellule dendritiche, le DLI, i linfociti tumore specifici, le CAR T.

## Vaccinazione antitumorale

La vaccinazione anti-tumorale impiega cellule dendritiche che insieme ai linfociti T citotossici sono responsabili della sorveglianza immunologica anti-tumorale (46-56). Le cellule dendritiche che possono essere rese inattive dal tessuto

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tumorale una volta raccolte con procedura aferetica possono essere espanse *in vitro* ed indotte a riconoscere antigeni tumorali diventando così nuovamente attive nei confronti del tessuto tumorale. In particolare, tali cellule possono scatenare l'attività citotossica T che può essere facilmente impiegata per valutare la risposta a protocolli di vaccino-terapia anti-tumorale. Questi, pur essendo sicuri e fattibili sono poco efficaci nei pazienti con LMC e leucemia acuta mieloide (LAM). Tuttavia, alcuni pazienti affetti da LAM hanno risposto alla vaccinazione con cellule dendritiche indotte a riconoscere l'antigene WT1 (Wilms Tumor 1). In questi pazienti la terapia con cellule dendritiche ibride autologhe impiegata come consolidamento dopo chemioterapia standard ha prolungato la durata della remissione. Pertanto, sono tuttora in corso protocolli di vaccinoterapia che impiegano cellule dendritiche educate verso vari antigeni tumorali dopo trapianto autologo o come terapia di consolidamento, mantenimento o re-induzione della remissione completa (RC). Inoltre, la vaccinoterapia con cellule dendritiche è stata impiegata anche in malattie linfoproliferative a cellule B. I risultati di uno studio di fase I che ha impiegato cellule dendritiche autologhe anti-idiotipo nel linfoma follicolare sono stati molto incoraggianti. Questi risultati sono stati confermati da altri studi condotti non solo nel linfoma follicolare ma anche in altri linfomi non-Hodgkin indolenti ed è stato riportato che lisati di leucemia acuta linfoblastica (LAL) a cellule B possono essere impiegati per generare cellule dendritiche da utilizzarsi in vaccinoterapia. Protocolli di vaccinoterapia con cellule dendritiche sono stati eseguiti anche nel mieloma multiplo. In questa patologia un protocollo di fase II condotto in pazienti con malattia non in remissione dopo trapianto autologo è risultato fattibile e ha permesso di migliorare la sopravvivenza media dei pazienti. Analoghi risultati sono stati riportati da altri studi che hanno dimostrato che la vaccinoterapia con cellule dendritiche è capace di ridurre la malattia minima residua nel mieloma multiplo.

## DLI

Linfociti T del donatore (DLI) possono essere raccolti mediante una procedura di aferesi seguita da una procedura di immuno-selezione. La sospensione cellulare così ottenuta sarà poi sottoposta a criopreservazione in modo che possa essere utilizzata qualora il paziente sviluppi una recidiva post-trapianto allogenico (57-64). Le DLI sono state per la prima volta impiegate nei pazienti con LMC in recidiva precoce dopo trapianto allogenico allo scopo di aumentare la chimera del donatore. La percentuale di risposta nei pazienti con LMC è >70%, ma in quelli con LAM, LAL e sindrome mielodisplastica (SMD) è <30%. È ormai stabilito che la dose massima di cellule CD3+ che possono essere infuse senza che si verifichi una GVHD debba essere $<1x10^8$/kg. Studi recenti hanno riportato una percentuale di risposta più alta (50-66%) nei pazienti con LAM e LAL che avevano ricevuto DLI in combinazione con chemioterapia, ma il follow-up mediano di questi pazienti era stato di soli 106 giorni e più del 60% aveva sviluppato una GVHD. L'infusione dei linfociti del donatore non aveva determinato un miglioramento della sopravvivenza ma si associava ad un effetto GVL nei pazienti recidivati dopo il trapianto. Talvolta

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le DLI causavano effetti collaterali inusuali come polimiosite, malattie autoimmuni e complicanze polmonari non infettive. Ancor più recentemente molti trials clinici hanno impiegato regimi di condizionamento ad intensità ridotta per ridurre la morbidità e mortalità peri-trapiantologica e DLI a scopo profilattico in una fase molto precoce del post-trapianto per sfruttare al massimo l'effetto GVL.

Un argomento tuttora discusso è il "timing" delle DLI. Alcuni studi hanno riportato una maggior incidenza di GVHD acuta quando le DLI vengono eseguite in fase precoce, mentre altri hanno indicato che la progressiva riduzione dell'immunosoppressione nell'arco di 6-8 settimane e la somministrazione in fase precoce delle DLI non comporta un aumento del rischio di GVHD acuta ma invece un aumentato rischio di GVHD cronica. Altri studi hanno inserito le DLI nel programma trapiantologico ed altre le hanno utilizzate nel trapianto TD per aumentare il chimerismo del donatore e ridurre l'incidenza di recidiva. In questo contesto le DLI determinavano una conversione della chimera da mista a completa nel 45% dei pazienti con un tasso di GVHD acuta del 33% simile a quello riportato da protocolli che non impiegavano le DLI. Queste ultime vengono impiegate anche nel trapianto ad intensità ridotta per potenziare la chimera del donatore con una percentuale di riuscita del 56%. L'efficacia delle DLI nel potenziare la chimera del donatore è stata analizzata anche dopo tecniche di espansione *in vitro*. È stato così dimostrato che queste DLI aumentano il rischio di GVHD acuta e cronica, riducono l'incidenza di recidiva e migliorano la sopravvivenza libera da malattia e da GVHD. Ci sono poi segnalazioni che suggeriscono cautela nella somministrazione di DLI ottenute da sospensioni di NK in pazienti con LAM ad alto rischio (65). Come abbiamo visto le cellule NK sono cellule del sistema immune innato che uccidono cellule tumorali senza che sia necessario l'"antigen primiing". La morte citotossica avviene a causa di uno sbilanciamento tra segnali attivatori ed inibitori mediati dai ligandi del KIR. Nonostante gli ottimi risultati riportati da studi condotti in vitro o in modelli sperimentali, l'infusione di cellule NK autologhe in pazienti con linfoma non ha fornito risultati incoraggianti forse perché le cellule tumorale erano diventate resistenti. Anche nelle LAM è stata osservata una debole azione citotossica delle cellule NK forse per una bassa densità dei recettori della citotossicità naturale. Le cellule NK allogeniche potrebbero possedere una maggiore attività, ma la preparazione di queste cellule richiede la rimozione dei linfociti T per ridurre il rischio di GVHD. In uno studio condotto in pazienti adulti affetti da LAM resistente/refrattaria l'infusione di NK del donatore in combinazione con IL2, fludarabina e ciclofosfamide ha consentito di ottenere una risposta nel 26% dei pazienti (66). L'efficacia terapeutica di questa procedura di trattamento dipende dalla densità dei recettori di citotossicità naturale per l'antigene e pertanto la riuscita di questo tipo di terapia sarà malattia specifica (67, 68).

## Linfociti tumore specifici

Linfociti citotossici (T linfociiti e cellule NK) possono essere isolati dal sangue periferico mediante aferesi e stimolati per essere preparati ad uccidere cellule tumorali (69-72). Queste cellule chiamate linfociti indotti ad uccidere mediante

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citokine (CIK) sono stati per la prima volta impiegati nel Mieloma Multiplo (MM). Un successivo studio aveva descritto un protocollo di "Good Clinical Practice" per la loro applicazione in trials clinici. Le cellule CIK possono essere raccolte al momento della raccolta di cellule staminali ematopoietiche in pazienti candidati a trapianto autologo, espanse *in vitro* con γ-interferon, anticorpo anti-CD3, IL2 ed usate nel post-trapianto come immunoterapia adottiva nei pazienti con linfoma in recidiva. Uno studio che aveva confrontato il decorso clinico di pazienti anziani con linfoma che avevano ricevuto IL2 in combinazione con CIK con quello di pazienti che avevano ricevuto IL2 da sola aveva riportato una migliore sopravvivenza per il primo gruppo di pazienti. Studi ancora più recenti hanno proposto l'impiego di CIK in combinazione con anticorpo monoclonale anti-CD20 come terapia di mantenimento nei pazienti con linfoma follicolare e l'impiego di CIK in combinazione con brentuximab nei pazienti con linfoma di Hodgkin in recidiva. L'impiego delle CIK nei pazienti con LAM in recidiva dopo trapianto era stato per la prima volta descritto da un piccolo studio che aveva impiegato anche le DLI. Un altro studio aveva utilizzato le CIK nel post-trapianto e aveva riportato che tre pazienti avevano ottenuto una RC della durata di circa un anno ma avevano sviluppato una GVHD. Un altro studio che aveva analizzato la combinazione cellule dendritiche, CIK e chemioterapia a basse dosi in pazienti anziani con LAM aveva mostrato un buona risposta ematologica.

**"Chimeric Antigen Receptor Cells" (CAR)**

Sono cellule T super-preparate ad uccidere e specificamente ingegnerizzate a riconoscere antigeni tumorali (73-90). Linfociti T periferici vengo raccolti e trasdotti ad esprimere un recettore della cellula T (TCR) che possiede un dominio extracellulare di legame specifico per un bersaglio tumorale. Per inserire il TCR chimerico vengono utilizzati metodi diversi come lentivirus, retrovirus ed elettroporazione. Le CAR T di prima generazione attaccavano l'antigene tumorale ad un dominio di segnalazione intracellulare CD3ζ attraverso un dominio trans-membrana. Le CAR T di seconda generazione avevano aggiunto regioni di co-stimolazione (ad esempio CD28, 4-1BBL) al recettore chimerico CD3ζ. Le CAR T di terza generazione hanno incorporato multipli domini di co-stimolazione (ad esempio CD28-OX40, CD28-4-1BBL). Studi ex vivo hanno dimostrato che l'efficacia antitumorale dei tre tipi di CAR è sovrapponibile, ma trials clinici hanno dimostrato una diversa persistenza con le CAR T di seconda generazione. In campo ematologico le CAR T di prima generazione erano state create per riconoscere l'antigene CD19 espresso da cellule di LAL-B, di Leucemia Linfatica Cronica (LLC) e di Linfomi non Hodgkin (LNH). Nel 2017 la "Food and Drug Administration" (FDA) aveva approvato l'impiego clinico di due tipi di CAR T nella LAL-B refrattaria/resistente. Successivamente, diversi trials clinici avevano dimostrato che la terapia con CAR T CD19 specifiche consentiva di raggiungere una RC nel 90% delle LAL in recidiva. La maggior complicanza di questa terapia era costituita dalla sindrome da liberazione delle citokine (CRS). Questo effetto avverso della terapia si manifestava con febbre, insufficienza d'organo e neuro-

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tossicità severa e fatale se non prontamente riconosciuta. Una migliore conoscenza della CRS ha portato all'impiego dell'anticorpo monoclonale tocilizumab nelle sindromi più severe. CAR T CD19 specifiche sono state impiegate anche in altri disordini linfoproliferativi CD19 positivi. Nelle LLC una RC è stata raggiunta nel 50% dei pazienti e nel 30% dei pazienti in recidiva post-trapianto. Nei bambini con LAL refrattaria/ resistente sono state impiegate CAR T "universali" prodotte cioè con procedure di manipolazione genetica (ad esempio *Crispr/Cas* e TALEN) per evitare la GVHD. Si tratta di una tecnologia eccitante visto che consente l'impiego di cellule T allogeniche che sono così sempre prontamente disponibili per l'esecuzione di una immunoterapia. Tuttavia, nonostante l'elevata percentuale di risposte le LAL a cellule B possono recidivare dopo terapia con CAR T. Una ripresa di malattia può avvenire per due diversi meccanismi: esaurimento progressivo delle cellule CAR T CD19 positive o recidive CD19 negative. Pertanto sono state sviluppate diverse strategie terapeutiche per risolvere questa problematica come l'impiego di antigeni tumorali alternativi (ad esempio CD22), di diversi antigeni tumorali (ad esempio combinando CD19 e CD22) in CAR T "tandem" o l'infusione di due tipi di CAR T. Gli ottimi risultati ottenuti nelle LAL dalle CAR T hanno portato all'estensione di questa tecnologia anche a LAM, al MM ed ai tumori solidi. Lo scarso successo delle CAR T nel MM ha portato alla ricerca di bersagli antigenici alternativi come CD138 e CS-1 che si sono dimostrati validi *in vitro*. Nelle LAM sono stati proposti diversi possibili bersagli come il CD123, l'antigene LeY, il recettore per i folati. La FDA ha sospeso la produzione delle CAR T CD123 positive per problemi di sicurezza. Nonostante questa battuta d'arresto le CART rappresentano un'immunoterapia antitumorale innovativa per la possibilità di sviluppare un tipo di CAR T per ogni tipo di neoplasia o di antigene tumorale. Inoltre, molto recentemente è stata valutata la possibilità di trasferire la tecnologia CAR alle cellule NK. Uno studio è riuscito ad indurre CAR NK CD19 positive che possedevano attività citolitica nei confronti di cellule di LLC resistente dalla linea cellulare NK-92 e ha dimostrato che per la generazione di questo tipo di CAR è più efficace la transfezione con un lentivirus piuttosto che l'elettroporazione. Altri studi hanno riportato l'efficacia delle cellule NK CAR CD19 positive nelle LAL-B e nel MM. Un vantaggio delle CAR NK è costituito dalla possibilità di avere un'immunoterapia rapidamente disponibile senza rischi di GVHD per pazienti con malattie linfoproliferative a cellule B in recidiva post-trapianto.

## Bibliografia

1. Im A, Pavletic MZ. Immunotherapy in hematologic malignancies: past present and future. Hematol Oncol. 2017; 10: 94.
2. Orti G, Barba P, Fox L, et al. Donor lymphocyte infusions in AML and MDS: enhancing the graft versus leukemia effect. Exp. Hematol. 2017; 48: 1-11.
3. Ferrara JL, Levine JE, Reddy P, et al. Graft versus host disease. Lancet 2009; 373: 1550-1561.
4. Praggs KS, Mackinnon S. Cellular therapies: donor lymphocyte infusions. Curr Opin in Hematol. 2001; 8: 349-354.

TORNA ALL'INDICE

5. Saad A, Lamb S. Ex vivo T cell depletion in allogeneic hematopoietic stem cell transplantation: past present and future. Bone Marr. Transpl. 2017; 52: 1241-1248.
6. Xia G, Truitt RL, Johnson BD: Graft-versus-leukemia and graft-versus-host reactions after after donor lymphocyte infusions are initiated host-antigen presenting cells and regulated by T regulatory cells in early and long-term chimeras. Biol Blood Marr. Transpl. 2006; 12: 397-407.
7. SehnL H, Aylea EP, Weller E, et al. Comparative outcomes of T cell-depleted and non-T cell-depleted allogeneic bone marrow transplantation for chronic myeloid leukemia: impact of donor lymphocyte infusions. J Clin Oncol. 1999; 17: 561-568.
8. Hobbs GS, Perales MA. Effects of T-cell depletion on allogeneic hematopoietic stem cell transplantation outcomes in AML patients. J Clin Med. 2015; 4: 488-503.
9. Ruggeri L, Parisi S, Urbani E, et al. Alloreactive natural killer cells for the treatment of acute myeloid leukemia: from stem cell transplantation to adoptive immunotherapy. Frontiers in Immunol. 2015; 6: 479.
10. Strigaris K, Barrett AJ. The importance of natural killer cell killer immunoglobulin-like receptor-mismatches in transplant outcomes. Curr Opin. Hematol. 2017; 24: 489.
11. Davies JOJ, Stringaris K, Barrett AJ. Opportunities and limitations of natural killer cells as adoptive therapy for malignant disease. Cytotherapy. 2014; 16: 1453.
12. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 199; 94: 333.
13. Ruggeri L, Mancusi A, Burchielli E, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. Blood Cell Mol Dis. 2008; 40: 84.
14. Ruggeri L, Mancusi A, Capanni M, et al: Donor natural killer cells allo-recognition of missing self in haplo-identical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. Blood. 2007; 110: 433.
15. Bruno B, Seguy D, Maunoury V, et al. Improvement of supportive care in patients undergoing myelo-ablative allogeneic stem cell transplantation not only reduces transplant-related mortality but also increases long-term survival. Blood. 2008; 112: 4318.
16. Saglio F, Hanley PJ, Bollard CM, the time is now: moving toward virus-specific T cells after allogeneic hematopoietic stem cell transplantation as the standard of care. Cytotherapy. 2014; 16: 149.
17. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T cell clones from the donor. New Engl. J Med. 1995; 333: 1038.
18. Peggs KS, Thomson K, Samuel E, et al. Directly selected cytomegalovirus-reactive donor T cells confer rapid and safe systemic reconstitution of virus-specific immunity following stem cell transplantation. Clin Infec Dis. 2011; 52: 49.

TORNA ALL'INDICE

19. Blyth E, Clancy L, Simms R, et al. Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic hematopoietic stem cell transplantation. Blood. 2013; 121: 3745.
20. Neuenhahn M, Albrecht J, Odendhal M, et al. Transfer of immunity manipulated CMV-specific T cells from stem cell or third party donors to treat CMV infection after allo-HSCT. Leuk. 2017; 31: 2161.
21. Cobbold M, Khan N, Pourgheysari B, et al. Adoptive transfer of cytomegalovirus specific T cells CTL to stem cell transplant patients after selection by HLA-peptide tetramers. J Exp Med. 2005; 202: 379.
22. Feuchtinger T, Matthes-Martin S, Richard C, et al. Safe adoptive transfer of virus-specific T cell immunity for adenovirus infection after allogeneic stem cell transplantation. Br J Haematol. 2006; 134: 164.
23. Feuchtinger T, Richard C, Joachim S, et al. Clinical-grade generation of hexon-specific T cells for adoptive T cell transfer as a treatment of adenovirus infection after allogeneic stem cell transplantation. J Immun. 2008; 31: 199.
24. Hemskerk B, van Vreeswijk T, Veltrop-Duits LA, et al. Adenovirus specific CD4+ T cell clones recognizing endogenous antigens inhibit viral replication in vitro through cognate interaction. J Immuol. 2006; 177; 8851.
25. Leen AM, Meyers GD, Bollard CM, et al. T-cell immunotherapy for adenoviral infection of stem cell transplant recipients. New York Aca. Sci. 2005; 1062: 104.
26. Heslop HE, Ne CY, Li C, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. Nat Med. 1996; 2: 551.
27. Gustafsson A, Levitsky V, Zou JZ, et al. Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop post-transplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T cells. Blood. 2000; 95: 807.
28. Comoli P, Basso S, Zecca M, et al. Preemptive therapy of EBV-related lymphoproliferative disease after pediatric haplo-identical stem cell transplantation. Am J Transpl. 2007; 7: 1048.
29. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010; 115; 925.
30. Doubrovina E, Oflaz-Sozmen B, Prockop SE, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic stem cell transplantation. Blood. 2012; 119: 2644.
31. Khanna N, Stuehler C, Conrad B, et al. Generation of multi-pathogen T cell product for adoptive immunotherapy based on activation-dependent expression of CD154. Blood. 2011; 118: 1121.
32. Leen MA, Christin A, Meyers GD, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infection after haplo-identical and matched unrelated stem cell transplantation. Blood. 2009; 114: 4283.

TORNA ALL'INDICE

33. Hanley PJ, Lam S, Shpall EJ, et al. Expanding cytotoxic T lymphocytes from umbilical cord blood that target cytomegalovirus, Epstein-Barr virus and adenovirus. J Vis Exp. 2012; 61: e3627.
34. Dotoli GM, De Sanctis GC, Orellana MD, et al. Mesenchymal stromal cell infusion to treat steroid-refractory acute GVHD III/IV after hematopoietic stem cell transplantation. Bone Marr Transpl. 2017; 52: 859.
35. Munneke JM, Spruit MJ, Cornelissen AS, et al. The potential of mesenchymal stromal cells as treatment for severe steroid-refractory acute graft-versus-host-disease: a critical review of the literature. Transplantation. 2016; 100: 2300.
36. Bernardo MF, Locatelli F: Mesenchymal stromal cells in hematopoietic stem cell transplantation. Methods Mol Biol. 2016; 1416: 3.
37. Hashmi S, Ahmed M, Murad MH, et al. Survival after mesenchymal stromal cell therapy in steroid-refractory acute GVHD: systematic review and meta-analysis. Lancet Hematol. 2016; 3: e45.
38. Zhao K, Lou R, Huang F, et al. Immunomodulation effects of mesenchymal stromal cells on acute GVHD after hematopoietic stem cell transplantation. Biol Blood and Marr Transpl. 2015; 21: 97.
39. Sanchez-Guijo F, Caballero-VelazquezT, Lopez-Villar O, et al. Sequential third-party mesenchymal stromal cell therapy for steroid-refractory acute GVHD. Biol Blood and Marr Transpl. 2014; 20: 1580.
40. Introna M, Lucchini G, Dander E, et al. Treatment of graft versus host disease with mesenchymal stromal cell: a phase I study on 40 adult and pediatric patients. Biol Blood and Marr Transpl. 2014; 20: 375.
41. Resnik IB, Barkats C, Shapira MY, et al. Treatment of severe steroid resistant acute GVHD with mesenchymal stromal cells (MSC). Am J Blood Res. 2013; 3: 225.
42. Ball LM, Bernardo ME, Roelofs H, et al. Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute GVHD. Br J Haematol. 2013; 163: 501.
43. Kebriaiei P, Isola I, Bahceci E, et al. Adult human mesenchymal stem cells added to corticosteroid treatment for AGVHD. Biol of Blood Marr Transpl. 2009; 15: 804.
44. von Bonin M, Stőzel F, Goedecke A, et al. Treatment of refractory aGVHD with third-party MSC expanded in platelet lysate-containing medium. Bone Marr Transpl. 2009; 43: 245.
45. Zhou H. Guo M, Bian C, et al. Efficacy of bone marrow derived mesenchymal stem cells in the treatment of sclerodermatous chronic GVHD: clinical report. Biol of Blood Marr Transpl. 2010; 16: 403.
46. Duncan C, Roddie H. Dendritic cell vaccines in acute myeloid leukemia. Best Pract Res Clin Hematol. 2008; 21: 521.
47. Galati D, Zanotta S. Hematologic neoplasms: dendritic cell vaccines in motion. Clin Immunol. 2017; 183: 181.
48. Pyzer AR, Avigan DE, Rosenblatt JJ. Clinical trials of dendritic cell-based cancer vaccines in hematologic malignancies. Hum Vaccin Immunother. 2014; 10: 3125.

TORNA ALL'INDICE

49. Westermann J, Kopp J, van Lessen A, et al. Vaccination with autologous non-irradiated dendritic cells in patients with bcr-abl chronic myeloid leukemia. Br J Haematol. 2007; 137: 297.
50. Kitawaki T, Kadowaki N, Fukunaga K, et al. Cross-priming of CD8(+) T cells in vivo by dendritic cells pulsed with autologous apoptotic leukemic cells in immunotherapy for elderly patients with acute myeloid leukemia. Exp. Haematol. 2011; 39: 424.
51. Van Tendeloo VF, Van De Velde A, Van Driessche A, et al. Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. Proc Natl Acad Sci USA. 2010; 107: 13624.
52. Rosenblatt J, Stone R, Uhl I, et al. Individualized vaccination of AML patients in remission is associated with induction of antileukemia immunity and prolonged remission. Sci Trans. Med. 2016; 8: 368ra171.
53. Hus I, Rolinski J, Tabarkiewicz J, et al. Allogeneic dendritic cells pulsed with tumor lysates or apoptotic bodies as immunotherapy for patients with early-stage B-cell chronic lymphocytic leukemia. Leukemia. 2005; 19: 1621.
54. Hus I, Schmitt M, Tabarkiewicz J, et al. Vaccination of B-CLL patients with autologous dendritic cells can change the frequency of leukemia antigen-specific CD8+ T cells as well as CD4+CD25+FoxP3+ regulatory T cells towards an antileukemia response. Leukemia. 2008; 22: 1007.
55. Lacy MQ, Mandrekar S, Dispenzieri A, et al. Idiotype-pulsed antigen-presenting cells following autologous transplantation for multiple myeloma may be associated with prolonged survival. Am J Hematol. 2009; 84: 799.
56. RosenblattJ, Avivi J, Vasir B, et al. Vaccination with dendritic cell/tumor fusions following autologous stem cell transplantation induces immunologic and clinical responses in multiple myeloma patients. Clin Cancer Res. 2013; 19: 3640.
57. Chalandon Y, Passweg JR, Schmid C, et al. Outcome of patients developing GVHD after DLI given to treat CML relapse: a study by the Chronic Leukemia Working Party of the EBMT. Bone Marr Transpl. 2010; 45: 558.
58. Portr DI, Antin JH. The graft versus leukemia effect of allogeneic cell therapy. Ann. Rev Med. 1999; 50: 369.
59. Bar M, Sandmaier BM, Inamoto Y, et al. Donor lymphocyte infusion for relapsed hematologic malignancies after allogeneic hematopoietic cell transplantation: prognostic relevance of the initial CD3+ cell dose. Biol Blood Marr Transpl. 2013; 19: 949.
60. Yergin Z, Őzkurt Z, Aki Ş, et al. Donor lymphocyte infusion for leukemia relapse after hematopoietic stem cell transplantation. Transfer Apher Sci. 2010; 42: 239.
61. Tan J, Wang Y, Yu SJ, et al. Prognostic factors on GVHD-free and relapse-free survival after allogeneic hematopoietic cell transplantation for adults with acute leukemia. Leuk Res. 2017; 59: 1.
62. Befting M, Halkes CJ, de Wreede LC, et al. Myelo-ablative T-cell depleted allo-SCT early sequential prophylactic donor lymphocyte infusion is an effi-

TORNA ALL'INDICE

cient and safe post-remission treatment for ALL. Bone Marr Transpl. 2014; 49: 287.
63. Marks DI, Lush R, Cavenagh J, et al. The toxicity and efficacy of donor lymphocyte infusions given after reduced-intensity conditioning allogeneic stem cell transplantation. Blood. 2002; 100: 3108.
64. Spitzer TR, McAfee S, Sackstein R, et al. Intentional induction of mixed chimerism and achievement of of antitumor responses after non-myeloablative conditioning therapy and HLA-matched donor bone marrow transplantation for refractory hematologic malignancies. Biol Blood Marr Transpl. 2000; 6: 309.
65. Rubnitz JE, Inaba H, Ribeiro RC, et al. NKAML: a pilot study to determine the safety and feasibility of haplo-identical natural killer cell transplantation in childhood acute myeloid leukemia. J Clin Oncol. 2010; 28: 955.
66. Miller SJ, Soignier Y, Panoskaltsis-Montari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005; 105: 3051.
67. Dahlberg CI, Sarhan D, Chrobok M, et al. Natural killer cell-based therapies targeting cancer: possible strategies to gain and sustain antitumor activity. Front Immunol. 2015; 6: 605.
68. Fang F, Xiao W, Tian Z, et al. NK cell-based immunotherapy for cancer. Semin Immunol. 2017; 31: 37.
69. Leivas A, Perez-Martinez A, Blanchard MJ, et al. Novel treatment strategies with autologous activate and expanded natural killer cells plus anti-myeloma drugs for multiple myeloma. Oncoimmunology. 2016; 5: e1250051.
70. Garrcia-Munoz R, Lopez-Diaz-de-Cerio A, Feliu J, et al. Follicular lymphoma: in vitro effects f combining lymphokine-activated killer (LAC) cell-induced cytotoxicity and rituximab- and obinutuzumab-dependent cellular cytotoxicity (ADCC) activity. Immunol. Res. 2018; 64: 548.
71. Esser L, Weiher H, Schmidt-Wolf I, et al. Increased efficacy of brentuximab vedotin (SGN-35) in combination with cytokine-induced killer cells in lymphoma. Int J Mol Sci. 2016; 17.
72. Rettinger E, Huenecke S, Bonig H, et al. Interleukin-15-activated cytokine-induced killer cells may sustain remission in leukemia patients after allogeneic stem cell transplantation: feasibility, safety and first insight on efficacy. Haematologica. 2016; 101: e153.
73. Landoni E, Savoldo B Treating hematological malignancies with cell therapy: where are we now? Expert Opin Biol Ther. 2017; 1-11.
74. Maude SL, Teachey DT, Porter DL, et al. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. Blood. 2015; 125: 4017.
75. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CART cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med. 2014; 6: 224a.
76. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor modified T cells for acute lymphoid leukemia. New Eng J Med. 2013; 368: 1509.

TORNA ALL'INDICE

77. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial Lancet. 2015; 385; 517.
78. Turtle CJ, Riddell SR, Maloney DG. CD19-targeted chimeric antigen receptor-modified T-cell immunotherapy for B-cell malignancies. Clin Pharmacol Ther. 2016; 100: 252.
79. Fitzgerlad JC, Weiss SL, Maude SL, et al. Cytokine release syndrome after chimeric antigen receptor T cell therapy for acute lymphoblastic leukemia. Crit Care Med. 2017; 45: e124.
80. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remission in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med. 2015; 7: 303ra139.
81. Kochenderfer JN, Dudley ME, Carpenter RO, et al. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood. 2013; 122: 4129.
82. Ruella M, Maus MV.Catch me if you can: leukemia escape after CD19-directed T cell immunotherapies. Comput Struct Biotech J. 2016; 14: 357.
83. Ruella M, Barrett DM, Kenderian SS, et al. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J Clin Invest. 2016; 126: 3814.
84. Chu J, He S, Deng Y, et al. Genetic modification of T cells redirected Toward CS1 enhances eradication of myeloma cells. Clin Cancer Res. 2014; 20; 3989.
85. Rosenzweig M, Urak R, Walter M, et al. Preclinical data support leveranging CS1 chimeric antigen receptor T cell therapy for systemic light chain amyloidosis. Cytotherapy. 2017; 19: 861.
86. Mardiros A, Dos Santos C, McDonald T, et al. T cells expressing CD123-specific chimeric antigen receptors exhibit specific cytolytic effector functions and antitumor effects against human acute myeloid leukemia. Blood. 2013; 122: 3138.
87. Ritchie DS, Neeson PJ, Khot A, et al Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther. 2013; 21: 2122.
88. Lynn RC, Poussin M, Kalota A, et al Targeting folate receptor β on acute myeloid leukemia blasts with chimeric antigen receptor-expressing T cells. Blood. 2015; 125: 3466.
89. Boissel L, Betancur M, Wels WS, et al. Transfection with mRNA for CD19specific chimeric antigen receptor restores NK cell mediated killing of CLL cells. Leuk Res. 2009; 33: 1255.
90. Boissel L, Betancur M, Lu W, et al. Comparison of mRNA and Lentiviral based transfection of natural killer cells with chimeric antigen receptors recognizing lymphoid antigens. Leuk Lymphoma. 2012; 53: 958.

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# 7

# Natural Killer cells, from bench to bedside: past, present and future

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During evolution, the innate immune system predated the development of adaptive immunity. Despite the acquisition of a more sophisticated defense system, the innate immunity still plays a major role in defenses against infections in contemporary vertebrates. In addition, the two systems co-evolved and are tightly integrated (1, 2). For example, different cell types of the innate immunity express Fc receptors specific for antibodies, i.e. products of the adaptive immunity. In addition, dendritic cells (DC), capable of antigen/pathogen capture, evolved towards highly specialized antigen-presenting cells that are strictly required for T cell activation and initiation of adaptive responses. In general, cells and soluble factors of the innate immunity provide early defenses against infections. In most instances (over 90% of infections), they allow clearance of the invading pathogens, keeping the infection at a subclinical level (3). Cells of the innate immunity express a number of receptors, the so called "pattern recognition receptors" (PRR), coded by non-rearranging genes that recognize conserved microbial structures absent in the host (referred to as "pathogen-associated molecular pattern" (PAMPs) that represent "danger signals". Cells of the innate immunity are represented by leukocytes including mast cells, different phagocytic cells (including macrophages, neutrophils and DC), basophils and eosinophils, natural killer cells and other recently identified "innate lymphoid cells" (ILC). NK cells are potent cytolytic cells capable of killing tumor and virus-infected cells. Human NK cells express an array of activating and inhibitory receptors (4, 5).

Innate lymphoid cells (ILC) represent a growing family of cells that are collectively involved in host protection against pathogens and regulation of tissue homeostasis. In humans, in addition to NK cells, also other mature ILC populations have been characterized. We could provide evidence that CD34+ cell precursors capable of developing into ILC3 (or NK cells) are present in decidua and in tonsils (6). The presence of both ILC3 and their precursors in given tissues may allow not only a rapid response of ILC3 to relevant stimuli (e.g. pathogens), but also a prompt proliferation/differentiation of precursors leading to a larger populations of effector cells. It is likely that hematopoietic precursors present in different mucosal tissues may give rise also to other mature ILC populations, including ILC1 and ILC2. Regarding the ILC function, different from NK cells, ILC1, ILC2 and ILC3 subsets are non-cytolytic (helper ILCs). They release different cytokines that mirror those



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produced by regulatory T cell-subsets including Th1, Th2 and Th17 (4). Notably, the type 2 cytokine-releasing ILC2, are thought to be involved in the initiation of allergic responses, possibly in response to IL-33, produced by mucosal epithelial cells triggered by allergens. Different from other ILC, NK cells are cytolytic and play a primary role in the first line of innate responses to viral infections and in the immunosurveillance against tumors.

NK cell function is regulated by inhibitory and activating receptors most of which identified by Alessandro Moretta. The main inhibitory receptors recognize HLA-cl I molecules (7). Importantly, killer Ig-like receptors recognize allotypic determinants shared by different HLA-cl I alleles, while CD94/NKG2A recognizes the non–classical HLA-E. The need of the NK cell inactivation, implied the existence of activating receptors. The prototypes and the most important in tumor cell killing were discovered in our lab. Named NKp46, NKp44, NKp30 according to their molecular weight, they were collectively called natural cytotoxicity receptors (NCR) (8). While in an autologous setting all NK cells express one or more receptors for self HLA-class I, in an allogeneic setting, it is possible that KIRs present on a subset of NK cells do not recognizes alleles expressed by allogeneic cells ("alloreactive" NK cells). Although, NK cells display a potent anti-tumor activity in vitro and are thought to participate in the immunosurveillance against tumors, the tumor microenvironment may sharply inhibit their effector function, primarily by downregulating the surface expression of activating receptors. We have recently shown that another inhibitory mechanism mediated by PD-1 - PD-L1 interaction may strongly compromise the NK cell-mediated tumor cell killing (9). Importantly, cytolytic activity can be restored by interrupting the PD-1 - PD-L1 axis with specific mAbs. These results have relevant implications for those tumors that have lost/downregulated the HLA-class I expression, thus escaping the CTL-mediated control. NK cell cytotoxicity has been exploited in the haploidentical hemopoietic stem cells transplantation (HSCT) setting to cure high-risk leukemias (applied when no HLA-compatible donors are available). The infusion of mega-doses of T-depleted CD34+ HSC allows an efficient engraftment with unfrequent, mild grade, GvHD. In the T-depleted, haplo-HSCT, in the absence of donor T lymphocytes, NK cells play a central role in the anti-leukemia effect (10). A more recent evolution of the manipulation strategy of haplo-HSCT, based on the infusion of TCR$\alpha\beta$- and CD19-depleted mononuclear cells (including mature donor NK cells and TCR$\gamma\delta^+$ T cells in addition to CD34+ cells) results in a prompt availability of effector cells resulting in a better protection against early leukemia relapses and GvHD. Indeed, this strategy, successfully applied by Franco Locatelli and our group, led to a further improvement of the clinical outcome of pediatric patients, with a 70% 5 years survival in both ALL and AML pediatric patients (11, 12). Overall, the haplo-HSCT, now applied in numerous centers in the world, has allowed to safe thousands of lives of patients with otherwise lethal leukemias.

Notably, NK cells may represent a suitable platform for novel therapeutic approaches such as CAR-engineered NK cells. In addition, the use of monoclonal antibodies against inhibitory checkpoints may unleash anti-tumor NK ell activity.

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## Bibliografia


1. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harbor symposia on quantitative biology. 1989; 54 Pt 1: 1-13.
2. Janeway CA Jr., Medzhitov R. Innate immune recognition. Annual review of immunology. 2002; 20: 197-216.
3. Parham P. Innate immunity: The unsung heroes. Nature. 2003; 423: 20.
4. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate Lymphoid Cells: 10 Years On. Cell. 2018; 174: 1054-166.
5. Moretta A, Bottino C, Mingari MC, Biassoni R, Moretta L. What is a natural killer cell? Nature immunology. 2002; 3: 6-8.
6. Montaldo E, Teixeira-Alves LG, Glatzer T, Durek P, Stervbo U, Hamann W, et al. Human RORgammat(+)CD34(+) cells are lineage-specified progenitors of group 3 RORgammat(+) innate lymphoid cells. Immunity. 2014; 41: 988-1000.
7. Moretta A, Bottino C, Vitale M, Pende D, Biassoni R, Mingari MC, et al. Receptors for HLA class-I molecules in human natural killer cells. Annual review of immunology. 1996; 14: 619-148.
8. Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annual review of immunology. 2001; 19: 197-223.
9. Pesce S, Greppi M, Tabellini G, Rampinelli F, Parolini S, Olive D, et al. Identification of a subset of human natural killer cells expressing high levels of programmed death 1: A phenotypic and functional characterization. The Journal of allergy and clinical immunology. 2017; 139: 335-346 e3.
10. Moretta L, Locatelli F, Pende D, Marcenaro E, Mingari MC, Moretta A. Killer Ig-like receptor-mediated control of natural killer cell alloreactivity in haploidentical hematopoietic stem cell transplantation. Blood. 2011; 117: 764-771.
11. Locatelli F, Merli P, Pagliara D, Li Pira G, Falco M, Pende D, et al. Outcome of children with acute leukemia given HLA-haploidentical HSCT after alphabeta T-cell and B-cell depletion. Blood. 2017; 130: 677-685.
12. Locatelli F, Pende D, Falco M, Della Chiesa M, Moretta A, Moretta L. NK Cells Mediate a Crucial Graft-versus-Leukemia Effect in Haploidentical-HSCT to Cure High-Risk Acute Leukemia. Trends in immunology. 2018; 39: 577-590.


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# 8

# Vent'anni dopo: l'immunoterapia anti-citochine nell'artrite reumatoide

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Il trattamento dell'artrite reumatoide (AR) si basa sull'impiego di farmaci in grado di modificarne sia il decorso clinico sia il danno anatomo-funzionale. Questi farmaci prendono il nome di *disease modifying anti-rheumatic drugs* (DMARDs). Ne esistono tre diverse tipologie (1):

1) *conventional synthetic* (cs) DMARDs: piccole molecole convenzionali come il methotrexate (MTX)
2) *targeted synthetic* (ts) DMARDs: piccole molecole ad selettiva come gli inibitori delle Janus-kinasi
3) *biologic* (b) DMARDs: grandi molecole ottenute mediante biotecnologie come anticorpi monoclonali (mAb) o proteine ricombinanti di fusione.

I bDMARDs in grado di neutralizzare l'effetto di citochine pro-infiammatorie hanno costituito il primo approccio *"targeted"* nell'AR (2) e risultano tuttora i più utilizzati (3). Il blocco del TNF-α, suggerito 30 anni fa dal fondamentale studio *in vitro* di Brennan et al. (4), è entrato nel nostro uso clinico ormai da 20 anni.

Due altre citochine pro-infiammatorie sono state impiegate in clinica come bersaglio terapeutico nell'AR: l'interleuchina-1 (IL-1) e l'IL-6. Il blocco funzionale dell'IL-6 è uno dei pilastri della terapia biologica dell'AR mentre l'inibizione dell'IL-1, mediante l'antagonista recettoriale anakinra, rientra ormai solo marginalmente nelle strategie terapeutiche di questa malattia. Anakinra, canakinumab (mAb anti-IL-1) o rilonacept (proteina di fusione ad azione recettoriale dimerica) sono soprattutto efficaci nell'artrite giovanile sistemica, nella malattia di Still dell'adulto ed in varie sindromi auto-infiammatorie (5).

## Inibitori del TNF-α (TNFi)

Sono 5 le molecole approvate per l'impiego clinico nell'AR: una per uso endovenoso (infliximab) e 4 sottocutaneo (etanercept, adalimumab, golimumab, certolizumab pegol).

A parte etnercept, proteina di fusione contenente il recettore solubile del TNF, tutti sono mAb o frammenti di anticorpi. Sono oggi disponibili anche molecole biosimilari di infliximab, etanercept e adalimumab. TNFi trovano oggi indicazione an-



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che nelle spondiloartriti sieronegative assili e periferiche, nella psoriasi e nell'idrosadenite suppurativa, nelle malattie infiammatorie croniche intestinali (solo mAb), nelle uveiti refrattarie non infettive e nell'artrite cronica giovanile poliarticolare.

*Efficacia clinica*

Il primo studio clinico controllato (RCT) di fase II con TNFi risale al 1994. In questo studio una singola infusione di infliximab fornì la prima prova di efficacia clinica del blocco di una citochina nell'AR (6). Studi immediatamente successivi hanno permesso di definire dosaggio e tempi di somministrazione sia di infliximab, sia di etanercept (8).

Le prime esperienze con TNFi si riferivano a popolazioni con malattia articolare grave e di lunga durata. Negli anni successivi, TNFi sono stati testati anche in pazienti con AR in fase precoce con risultati molto positivi (9, 10). Tutti i TNFi hanno maggiore efficacia se combinati con MTX o altri csDMARD (11-13). Anche il massimo effetto sull'arresto del danno strutturale si osserva con il trattamento combinato TNFi-MTX (14, 15).

I TNFi hanno dimostrato di essere efficaci in una grande porzione di pazienti negli RCT; nella pratica clinica gli insuccessi primari e secondari possono tuttavia interessare oltre un terzo dei soggetti (16, 17). L'efficacia è simile ma esistono differenze di struttura, sito d'azione e dosaggio tra I vari TNFi. In presenza di risposta non adeguata con un primo TNFi, può essere utilizzato un bDMARD con diverso meccanismo d'azione oppure un altro TNFi (1). L'uso sequenziale di TNFi ha fornito risultati positivi in gran parte degli RCT (18-21) e in alcuni registri (22, 23). La comparsa di anticorpi anti-farmaco può condizionare una perdita di efficacia nel tempo (inefficacia secondaria). La terapia combinata con MTX riduce l'incidenza di immunogenicità (24).

*Profilo di sicurezza*

Nel registro LORHEN, le sospensioni del trattamento per eventi avversi avvicinavano quelle per inefficacia (25).

Alcune infezioni, talora opportunistiche, sono state registrate con maggiore frequenza rispetto agli RCT. L'incidenza di infezioni gravi risulta simile tra i diversi registri e stabile nel tempo (26, 27). Le più frequenti sono infezioni batteriche della cute e delle basse vie respiratorie anche se la frequenza di polmonite è elevata anche in AR non trattate con TNFi (28, 29). Fattori di rischio sono l'età, la VES e l'uso concomitante di corticosteroidi a dosi medio-alte (29).

I TNFi sono associati a riattivazione di tubercolosi latente (30). Il TNF aumenta l'attività macrofagica ed è coinvolto nel mantenimento dei granulomi (31, 32). La profilassi con un regime anti-tubercolare standard ha dimostrato di prevenire la riattivazione in corso di terapia con TNFi (33, 34).

Tra le infezioni latenti, quella da HBV rappresenta un problema frequente nel nostro Paese (35, 36). Per i pazienti HBsAg-positivi, la terapia antivirale deve essere iniziata prima di qualsiasi terapia con bDMARD, mentre per i pazienti con infezione da HBV risolta o occulta, si raccomanda un regolare monitoraggio (37).

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I maggiori registri hanno mostrato che l'incidenza complessiva di neoplasie in corso di TNFi non è dissimile da quella della popolazione generale o dei pazienti con AR trattati con csDMARD (38-40). Un aumentato rischio di linfoma e di *non melanoma skin cancer* è associato alla AR severa indipendentemente dal trattamento (41, 42). Un singolo studio ha indicato un aumentato rischio di melanoma (43).

La comparsa di auto-anticorpi (antinucleo, anti-DNA, anti-fosfolipidi etc.) a seguito di trattamento con TNFi è un evento frequente ma non riveste particolare rilievo clinico (44, 45).

Le comorbidità cardiovascolari sono associate all'attività della AR (46, 47). Dall'analisi del registro britannico, il trattamento con TNFi ha ridotto il rischio di infarto miocardico (48).

## Inibitori dell'IL6

IL-6 è una citochina pleiotropica che riveste un ruolo chiave nello sviluppo e nella progressione dell'AR (49, 50). IL-6 svolge le sue funzioni legandosi al proprio recettore (IL-6R) di membrana (*classic signalling*) o alla forma solubile dell'IL-6R (*trans-signalling*). Esiste inoltre la possibilità di presentazione alle cellule T attraverso IL-6R di membrana già complessato (*trans-presentation*). Gli anticorpi anti-IL6 sono in grado di bloccare le prime due vie mentre gli anti-IL-6R sono in grado di bloccarle tutte e tre (51).

Attualmente sono disponibili per uso clinico due bDMARDs, tocilizumab e sarilumab, entrambi mAb diretti contro IL-6R. L'impiego di tocilizumab trova oggi applicazione anche nella malattia di Castleman, nell'arterite giganto-cellulare e nella sindrome da rilascio citochinico indotta dalla terapia con CAR T-cells.

*Efficacia clinica e profilo di sicurezza*

Molti degli aspetti relativi all'impiego clinico degli anti-IL-6R nella AR sono analoghi per indicazioni, efficacia sul danno strutturale e rischio infettivo a quanto osservato con TNFi (52). L'efficacia in monoterapia di tocilizumab e sarilumab è superiore a quella di adalimumab (54, 55) ma probabilmente non di etanercept (56). Nella pratica clinica, l'impossibilità di associare MTX è un fattore di scelta di tocilizumab (57). Il trattamento con anti-IL-6R è associato ad un verticale calo della proteina C-reattiva (PCR), al miglioramento dell'anemia (58), della qualità del sonno e di eventuali aspetti depressivi (59) e anche del potenziale rischio aritmico connesso con stati infiammatori (60). Un recente studio (53) ha mostrato che l'impiego di tocilizumab come primo approccio farmacologico possa portare a percentuali di remissione superiori all'80%.

Sul piano della tollerabilità, rispetto al trattamento con TNFi, è segnalato un aumentato rischio di perforazioni intestinali, di neutropenia e di ipertransaminasemia; inoltre i bassi valori di PCR possono mascherare eventuali infezioni concomitanti (61). Il profilo lipidico che può risultare alterato in senso ipercolesterolemico (62). In realtà il profilo di rischio cardiovascolare complessivo risulta migliorato dal trattamento (63).

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## Prospettive future

Altre citochine pro-infiammatorie sono state testate o sono attualmente sotto valutazione come target terapeutico da parte di farmaci biologici nell'AR. Il blocco della IL-17, con secukinumab, si è dimostrato molto efficace nella psoriasi e nelle spondiloartriti ma non nell'AR, nonostante i promettenti dati sperimentali (64). L'inibizione del GM-CSF con mavrilimumab si sta rivelando efficace e potrebbe costituire una opzione terapeutica a breve (65).

Aspettative vengono riposte nella possibilità di ingegnerizzazione di alcuni farmaci biologici, siano essi immunoglobuline complete o frammenti immunoglobulinici. Un esempio molto semplice già in uso è costituito dalla coniugazione del frammento Fab2 anti-TNF con polietilene glicole. Questo farmaco (certolizumab pegol) ha la particolarità di non attraversare la placenta e quindi di essere utilizzabile con sicurezza in gravidanza (66).

Oltre all'approccio attuale che riguarda il blocco o la rimozione di proteine bersaglio biologicamente attive, nuove categorie di prodotti anticorpali o simil-anticorpali ingegnerizzati e armati (67) potrebbero prefigurare l'implementazione di strategie molecolari alternative in grado di indurre immunosoppressione o tolleranza nei siti di infiammazione cronica.

## Bibliografia

1. Smolen JS, Landewé R, Bijlsma J, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Ann Rheum Dis. 2017; 76: 960-977.
2. Emery P Reginster JY, Appelboom T, et al. WHO Collaborating Centre consensus meeting on anti-cytokine therapy in rheumatoid arthritis. Rheumatology. 2001; 40: 699-702.
3. Caporali R, Crepaldi G, Codullo V, et al. Twenty years of experience with tumour necrosis factor inhibitors: what have we learned? Rheumatology. 2018; 57: vii5-vii10.
4. Brennan FM, Chantry D, Jackson A, Maini R, Feldmann M. Inhibitory effect of TNF-α antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. Lancet. 1989; 2: 244-247.
5. Federici S, Martini A, Gattorno M. The Central Role of Anti-IL-1 Blockade in the Treatment of Monogenic and Multi-Factorial Autoinflammatory Diseases. Front Immunol. 2013; 31: 4, 351.
6. Elliott MJ, Maini RN, Feldmann M, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet. 1994; 344: 1105-1110.
7. Maini R, Clair EWS, Breedveld F, et al. Infliximab (chimeric anti-tumour necrosis factor a monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. Lancet. 1999; 354: 1932-1939.
8. Weinblatt ME, Kremer JM, Bankhurst AD, et al. A trial of etanercept, a

TORNA ALL'INDICE

recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med. 1999; 340: 25-39.
9. Goekoop-Ruiterman YPM, De Vries-Bouwstra JK, Allaart CF, et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. Arthritis Rheum. 2005; 52: 3381-3390.
10. Smolen JS, Emery P, Fleischmann R, et al. Adjustment of therapy in rheumatoid arthritis on the basis of achievement of stable low disease activity with adalimumab plus methotrexate or methotrexate alone: the randomised controlled OPTIMA trial. Lancet. 2014; 383: 321-332.
11. Burmester GR, Mariette X, Montecucco C et al. Adalimumab alone and in combination with disease-modifying antirheumatic drugs for the treatment of rheumatoid arthritis in clinical practice: the Research in Active Rheumatoid Arthritis (ReAct) trial. Ann Rheum Dis. 2007; 66: 732-739.
12. Stefano RD, Frati E, Nargi F, et al. Comparison of combination therapies in the treatment of rheumatoid arthritis: leflunomide-anti-TNF-alpha versus methotrexate-antiTNF-α. Clin Rheumatol. 2010; 29: 517-524.
13. Weinblatt ME, Keystone EC, Furst DE, et al. Adalimumab, a fully human antitumor necrosis factor a monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum. 2003; 48: 3545.
14. Breedveld FC, Weisman MH, Kavanaugh AF, et al. The PREMIER study: a multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum. 2006; 54: 2637.
15. Klareskog L, van der Heijde D, de Jager JP, et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet. 2004; 363: 67581.
16. Weaver AL, Lautzenheiser RL, Schiff MH, et al. Real-world effectiveness of select biologic and DMARD monotherapy and combination therapy in the treatment of rheumatoid arthritis: results from the RADIUS observational registry. Curr Med Res Opin. 2006; 22: 18598.
17. Hyrich KL, Watson KD, Silman AJ, Symmons DPM. Predictors of response to anti-TNF-alpha therapy among patients with rheumatoid arthritis: results from the British Society for Rheumatology Biologics Register. Rheumatology. 2006; 45: 155865.
18. Smolen JS, Kay J, Matteson EL, et al. Insights into the efficacy of golimumab plus methotrexate in patients with active rheumatoid arthritis who discontinued prior antitumour necrosis factor therapy: post-hoc analyses from the GO-AFTER study. Ann Rheum Dis. 2014; 73: 1811-1818.
19. Schoels M, Aletaha D, Smolen JS, Wong JB. Comparative effectiveness and safety of biological treatment options after tumour necrosis factor a inhibitor

TORNA ALL'INDICE

failure in rheumatoid arthritis: systematic review and indirect pairwise meta-analysis. Ann Rheum Dis. 2012; 71: 130-138.
20. Manders SH, Kievit W, Adang E, et al. Cost-effectiveness of abatacept, rituximab, and TNFi treatment after previous failure with TNFi treatment in rheumatoid arthritis: a pragmatic multi-centre randomised trial. Arthritis Res Ther. 2015; 17: 134.
21. Gottenberg JE, Brocq O, Perdriger A, et al, Non-TNF-Targeted Biologic vs a Second Anti-TNF Drug to Treat Rheumatoid Arthritis in Patients With Insufficient Response to a First Anti-TNF Drug: A Randomized Clinical Trial. JAMA. 2016; 316: 1172-1180.
22. Caporali R, Sarzi-Puttini P, Atzeni F, et al. Switching TNFalpha antagonists in rheumatoid arthritis: the experience of the LORHEN registry. Autoimmun Rev. 2010; 9: 46-59.
23. Codullo V, Iannone F, Sinigaglia L, et al. Comparison of efficacy of first- versus second-line adalimumab in patients with rheumatoid arthritis: experience of the Italian biologics registries. Clin Exp Rheumatol. 2017; 35: 660-665.
24. Burmester G-R, Kivitz AJ, Kupper H, et al. Efficacy and safety of ascending methotrexate dose in combination with adalimumab: the randomised CONCERTO trial. Ann Rheum Dis. 2015; 74: 1037-1044.
25. Marchesoni A, Zaccara E, Gorla R, et al. TNF-α antagonist survival rate in a cohort of rheumatoid arthritis patients observed under conditions of standard clinical practice Ann N Y Acad Sci. 2009; 1173: 837-846.
26. Listing J, Strangfeld A, Kary S, et al. Infections in patients with rheumatoid arthritis treated with biologic agents. Arthritis Rheum. 2005; 52: 3403-12.
27. Atzeni F, Sarzi-Puttini P, Botsios C, et al. Long-term antiTNF therapy and the risk of serious infections in a cohort of patients with rheumatoid arthritis: comparison of adalimumab, etanercept and infliximab in the GISEA registry. Autoimmun Rev. 2012; 12: 22-59.
28. Wolfe F, Caplan L, Michaud K. Treatment for rheumatoid arthritis and the risk of hospitalization for pneumonia: associations with prednisone, disease-modifying antirheumatic drugs, and anti-tumor necrosis factor therapy. Arthritis Rheum. 2006; 54: 628-634.
29. Favalli EG, Desiati F, Atzeni F, et al. Serious infections during anti-TNFa treatment in rheumatoid arthritis patients. Autoimmun Rev. 2009; 8: 266-273.
30. Gomez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum. 2003; 48: 212-227.
31. Kindler V, Sappino AP, Grau GE, Piguet PF, Vassalli P. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell. 1989; 56: 731-740.
32. Bekker L-G, Freeman S, Murray PJ, Ryffel B, Kaplan G. TNF-a controls intracellular mycobacterial growth by both inducible nitric oxide synthase-dependent and inducible nitric oxide synthase-independent pathways. J Immunol. 2001; 166: 6728-6734.

TORNA ALL'INDICE

33. Zhang Z, Fan W, Yang G et al. Risk of tuberculosis in patients treated with TNF-α antagonists: a systematic review and meta-analysis of randomised controlled trials. BMJ Open. 2017; 7: e012567.
34. Carmona L, Gomez-Reino JJ, Rodrı´guez-Valverde V, et al. Effectiveness of recommendations to prevent reactivation of latent tuberculosis infection in patients treated with tumor necrosis factor antagonists. Arthritis Rheum. 2005; 52: 1766-1772.
35. Dougados M, Soubrier M, Antunez A, et al Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA). Ann Rheum Dis. 2014; 73: 62-68.
36. Caporali R, Bobbio-Pallavicini F, Atzeni F, Sakellariou G, Caprioli M, Montecucco C. Safety of tumor necrosis factor alpha blockers in hepatitis B virus occult carriers (hepatitis B surface antigen negative/anti-hepatitis B core antigen positive) with rheumatic diseases. Arthritis Care Res. 2010; 62: 749-754.
37. Nard DF, Todoerti M, Grosso V, et al. Risk of hepatitis B virus reactivation in rheumatoid arthritis patients undergoing biologic treatment: extending perspective from old to newer drugs. World J Hepatol. 2015; 7: 344.
38. Strangfeld A, Hierse F, Rau R, et al. Risk of incident or recurrent malignancies among patients with rheumatoid arthritis exposed to biologic therapy in the German biologics register RABBIT. Arthritis Res Ther. 2010; 12: R5.
39. Bobbio-Pallavicini F, Caporali R, Sarzi-Puttini P, et al. Tumour necrosis factor antagonist therapy and cancer development: analysis of the LORHEN registry. Autoimmun Rev. 2010; 9: 175-180.
40. Ramiro S, Sepriano A, Chatzidionysiou K, et al. Safety of synthetic and biological DMARDs: a systematic literature review informing the 2016 update of the EULAR recommendations for management of rheumatoid arthritis. Ann Rheum Dis. 2017; 76: 110136.
41. Askling J, Fored C, Baecklund E et al. Haematopoietic malignancies in rheumatoid arthritis: lymphoma risk and characteristics after exposure to tumour necrosis factor antagonists. Ann Rheum Dis. 2005; 64: 1414.
42. Raaschou P, Simard JF, Asker Hagelberg C, Askling J. Rheumatoid arthritis, anti-tumour necrosis factor treatment, and risk of squamous cell and basal cell skin cancer: cohort study based on nationwide prospectively recorded data from Sweden. BMJ. 2016; 352: i262.
43. Raaschou P, Simard JF, Holmqvist M, Askling J. Rheumatoid arthritis, anti-tumour necrosis factor therapy, and risk of malignant melanoma: nationwide population based prospective cohort study from Sweden. BMJ. 2013; 346: f1939.
44. Bobbio-Pallavicini F, Alpini C, Caporali R, Avalle S, Bugatti S, Montecucco C. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. Arthritis Res Ther. 2004; 6: R264-272.
45. Cavazzana I, Bobbio-Pallavicini F, Franceschini F et al. Anti-TNF-alpha treatment in rheumatoid arthritis with anti-Ro/SSA antibodies. Analysis of 17 cases among a cohort of 322 treated patients. Clin Exp Rheumatol. 2007; 25: 676-683.

TORNA ALL'INDICE

46. Arts EEA, Fransen J, den Broeder AA, Popa CD, van Riel PLCM. The effect of disease duration and disease activity on the risk of cardiovascular disease in rheumatoid arthritis patients. Ann Rheum Dis. 2015; 74: 9981-10003.
47. Crepaldi G, Scirè CA, Carrara G, et al. Cardiovascular comorbidities relate more than others with disease activity in rheumatoid arthritis. PLoS One. 2016; 11: e0146991.
48. Low ASL, Symmons DPM, Lunt M, et al. Relationship between exposure to tumour necrosis factor inhibitor therapy and incidence and severity of myocardial infarction in patients with rheumatoid arthritis. Ann Rheum Dis. 2017; 76: 654-660.
49. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat. Immunol. 2015; 16, 448-457.
50. Dayer JM, Choy EHS. Therapeutic targets in rheumatoid arthritis: the interleukin-6 receptor. Rheumatology. 2010; 49: 15-24.
51. Garbers *C*, Heink *S*, Korn T, Rose-John S. Interleukin-6: designing specific therapeutics for a complex cytokine. Nat Rev Drug Discov. 2018; 17: 395.
52. Smolen JS, Shoels MM, Nishimoto N et al. Consensus statement on blocking the effects of interleukin-6 and in particular by interleukin-6 receptor inhibition in rheumatoid arthritis and other inflammatory conditions. Ann Rheum Dis. 2013; 72: 482-492.
53. Bijlsma JWJ, Welsing PMJ, Woodworth TG, et al. Early rheumatoid arthritis treated with tocilizumab, methotrexate, or their combination (U-Act-Early): a multicentre, randomised, double-blind, double-dummy strategy trial. Lancet. 2016; 388: 343-355.
54. Gabay C, Emery P, van Vollenhoven R, et al. Tocilizumab monotherapy versus adalimumab monotherapy for treatment of rheumatoid arthritis (ADACTA): a randomised, double-blind, controlled phase 4 trial. Lancet. 2013; 381: 1541-1550.
55. Burmester GR, Lin Y, Patel R, et al. Efficacy and safety of sarilumab monotherapy versus adalimumab monotherapy for the treatment of patients with active rheumatoid arthritis (MONARCH): a randomised, double-blind, parallel-group phase III trial. Ann Rheum Dis. 2017; 76: 840-847.
56. Tarp S, Furst DE, Dossing A, et al. Defining the optimal biological monotherapy in rheumatoid arthritis: a systematic review and meta-analysis of randomised trials. Semin Arthritis Rheum. 2017; 46: 699708.
57. Monti S, Klersy C, Gorla R, et al. Factors influencing the choice of first- and second-line biologic therapy for the treatment of rheumatoid arthritis: real-life data from the Italian LORHEN Registry. Clin Rheumatol. 2017; 36: 753-761.
58. Paul SK, Montvida O, Best JH, Gale S, Pethoe-Schramm A, Sarsour K. Effectiveness of biologic and non-biologic antirheumatic drugs on anaemia markers in 153,788 patients with rheumatoid arthritis: New evidence from real-world data. Semin Arthritis Rheum. 2018; 47: 478-484.
59. Choy EHS, Calabrese LH. Neuroendocrine and neurophysiological effects of interleukin 6 in rheumatoid arthritis. Rheumatology. 2018; 57: 1885-1895.
60. Lazzerini PE, Acampa M, Capecchi PL, et al. Antiarrhythmic potential of an-

TORNA ALL'INDICE

ticytokine therapy in rheumatoid arthritis: tocilizumab reduces corrected QT interval by controlling systemic inflammation. Arthritis Care Res. 2015; 67: 332-339.
61. Ogata A, Kato Y, Higa S, Yoshizaki K. IL-6 inhibitor for the treatment of rheumatoid arthritis: A comprehensive review. Mod Rheumatol. 2019; 29: 258-267.
62. Strang, A. C. Pro-atherogenic lipid changes and decreased hepatic LDL receptor expression by tocilizumab in rheumatoid arthritis. Atherosclerosis. 2013; 229: 174-181.
63. McInnes IB, Thompson L, Giles JT, et al. Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: MEASURE, a randomised, placebo-controlled study. Ann Rheum Dis. 2015; 74: 694-702.
64. Roeleveld DM, Koenders MI. The role of the Th17 cytokines IL-17 and IL-22 in Rheumatoid Arthritis pathogenesis and developments in cytokine immunotherapy. Cytokine. 2015; 74: 101-107.
65. Burmester GR, McInnes IB, Kremer JM, et al. Mavrilimumab, a Fully Human Granulocyte-Macrophage Colony-Stimulating Factor Receptor α Monoclonal Antibody: Long-Term Safety and Efficacy in Patients With Rheumatoid Arthritis. Arthritis Rheumatol. 2018; 70: 679-689.
66. Clowse MEB, Scheuerle AE, Chambers C, et al. Pregnancy Outcomes After Exposure to Certolizumab Pegol: Updated Results From a Pharmacovigilance Safety Database. Arthritis Rheumatol. 2018; 70: 1399-1407.
67. Schmid AS, Neri D. Advances in antibody engineering for rheumatic diseases. Nat Rev Rheumatol. 2019; 15: 197-207.

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# 9

# Terapie anti-T e -B linfocitarie in reumatologia

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The immune system has evolved to discriminate between self and non-self, defending the host against harmful microorganisms without attacking self-tissues. Several regulatory mechanisms control autoreactivity and are responsible for the maintenance of peripheral tolerance (1). Failure of these mechanisms can lead to a breakdown in tolerance, resulting in the development of autoimmunity. In autoimmune rheumatic diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), autoreactive T cells and B cells are activated as a consequence of defective immune regulation; these cells proliferate and differentiate into pathogenic T cells and B cells that induce inflammation and tissue damage through several mechanisms. Aim of the present brief discussion is to provide an overview of the major roles played by T and B lymphocytes and their interaction in autoimmune diseases, and of their possible targeting for therapeutic purposes.

## T cells that help B cells

CD4+ T cells play a critical role in stimulating effective B cell responses and production of high-affinity antibodies. T follicular helper (Tfh) cells are generally considered the dominant T cell population capable of providing help to B cells (2). The interactions between Tfh cells and B cells within follicles of secondary lymphoid organs (SLOs) occur with precise spatial and temporal coordination to yield productive antibody responses. In pathologic immune responses, T cell-B cell interactions also occur outside of SLOs within chronically inflamed peripheral tissues, which frequently develop aggregates of lymphocytes that promote B cell responses locally (3). Widespread recognition of the importance of T cell/B cell collaboration in driving immune-mediated pathology came from a landmark paper in 2009 (4) linking overproduction of Tfh with systemic autoimmunity. This work focused on sanroque mice which have a mutation in the E3 ubiquitin ligase Roquin-1 that regulates mRNA stability and is required for appropriate repression of ICOS expression, which regulates germinal centre (GC) activity. Mice with the Roquin mutation exhibited high ICOS expression, excessive Tfh formation and lupus-like pathology.

T cell/B cell collaboration occurs through several key pathways that can be targeted for therapeutic purposes:



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*CD40/CD40L*

CD40 and CD40L have long been recognized as key players in humoral immunity and are essential for GC formation (5,6). Blockade of CD40L signaling during an ongoing GC reaction abrogates the response, emphasizing the need for continuous CD40-CD40L interactions throughout the GC lifespan (7).

*CD28/CTLA-4*

Strength of T cell CD28 engagement influences Tfh differentiation. In mice that are deficient in CD28 signaling, T cells fail to form Tfh (8). The CTLA-4 pathway restricts the formation of Tfh by limiting T cell CD28 engagement (9) and CTLA-4 expression in the regulatory T cell compartment is essential for this process (10). Accordingly, deficiency or blockade of CTLA-4 in mice leads to hyper-engagement of CD28, overproduction of Tfh and spontaneous GC formation (9).

*OX40*

The ability of CD28 to promote Tfh development may reflect its capacity to upregulate secondary costimulatory receptors such as OX40 and ICOS. CD28 engagement triggers T cell OX40 upregulation (11) and ligation of OX40 in turn promotes CXCR5 expression on B cells, leading to their migration to follicles (12). Mice expressing OX40L constitutively on dendritic cells show increased numbers of CD4 T cells in their B cell follicles (13), and conversely deficiency (14) or blockade (15) of OX40 reduce Tfh numbers after viral challenge.

*ICOS*

ICOS is known to be required for the GC response (16) and its engagement promotes the differentiation and maintenance of Tfh cells (17). The level of ICOS upregulation on T cells undergoing activation in vivo is tightly coupled to the level of CD28 engagement (9) consistent with the idea that CD28 may promote GC formation via the ICOS pathway. ICOS is superior to CD28 in its capacity to activate phosphoinositide 3-kinase which is known to be required for Tfh cell differentiation and GC formation (18).

## T cell targeting

The central role of co-stimulation in T cell function makes it a promising target for drugs to modulate the function of T cells. First studies using a soluble CD28 protein to block co-stimulation, however, were ineffective due to a low affinity of CD28for its ligands. Abatacept (CTLA-4Ig), in contrast, represents a soluble, recombinant, fully humanized fusion protein, comprising the extracellular domain of CTLA-4 and the Fc portion of IgG1 which has been modified to reduce the Fc region capacity to induce antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Abatacept is the first biological compound that primarily aims to modulate T-cell activation in chronic inflammatory diseases such as RA. This effect is thought to be mediated by binding of abatacept

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to the costimulatory molecules CD80 and CD86 on APC, thereby blocking interaction with CD28 on T cells. In this manner autoreactive CD4+ T cells receive signal one in the absence of signal two which leads to a state of T-cell anergy or unresponsiveness.

## B cells

B cells are multifunctional lymphocytes that contribute to the pathogenesis of autoimmune diseases via B cell-intrinsic, antibody-mediated and T cell-dependent mechanisms. Although antibody production by B cells promotes both ADCC and CDC, B cells can also present antigen and provide T cell help (19). B cell activation and effector functions are regulated by immune checkpoints, including activating and inhibitory checkpoints. B cell functions are critical for orchestrating pathogenic immune responses, and thereby, B cells and B cell immune checkpoints represent promising therapeutic targets for autoimmune rheumatic disease. Two signals are required for the activation of B cells: the engagement of the B-cell receptor (BCR) and a co-stimulatory signal. Stimulatory checkpoints consist of numerous cell receptors and cytokines:

*CD40/CD40L*

Cognate T cells promote B cell and plasma cell differentiation by providing costimulatory signals in the form of CD40 ligand. These cognate T cells are reciprocally activated by engagement of CD40L with CD40 on the surface of B cells (20).

*Toll- like receptors*

As an alternative to CD40-mediated co-stimulation of BCRs, B cells can be activated independently of T cells via dual stimulation of the BCR and TLRs. TLRs recognize pathogen- associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that are important for host defence and wound healing.

*CD19*

B cell activation can be facilitated by the activation of the co-receptor CD19. CD19 is an immunoglobulin superfamily glycoprotein associated with the BCR and is expressed on B cells from the pre-B cell stage through to the plasma cell differentiation stage. CD19 signals though the tyrosine kinases LYN and phosphoinositide 3-kinase (PI3K), which amplify signals from the BCR, decreasing the threshold for BCR activation (21).

*BAFF*

B cell activating factor (BAFF; also known as TNFSF13B) is a cytokine that belongs to the TNF family and can facilitate B cell activation indirectly by promoting B cell survival, proliferation and/or differentiation (22).

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*IL-6*

The cytokine IL-6 was originally identified as a B cell growth factor and plasma cell differentiation factor, but IL-6 can also have pleiotropic effects on other immune cell types (23).

*IL-21*

The cytokine IL-21 is produced by multiple T helper cell subsets and has critical functions in B cell activation, proliferation, differentiation, affinity maturation and antibody production. IL-21 drives pro-inflammatory responses by promoting B cell activation and expansion, and patients with SLE, type 1 diabetes or inflammatory bowel diseases have increased serum concentrations of IL-21 compared with healthy individuals (24).

## B cell targeting

Cell depletion was an initial approach used to target B cells for the treatment of autoimmune disease. The B cell- depleting anti- CD20 antibody rituximab is FDA-approved for the treatment of RA. Although not approved for multiple sclerosis and having failed trials in SLE, rituximab is also used off- label for the treatment of these diseases on the basis of clinician experience (25). In addition, ocrelizumab, a humanized anti-CD20 monoclonal antibody, was approved in 2017 for the treatment of multiple sclerosis (26). Clinical studies have shown some efficacy of ocrelizumab in treating SLE (27), although concerns over adverse events have halted clinical trials for SLE and RA, and future studies will probably demonstrate its utility in other B cell- mediated diseases. Despite depleting only B cells that express CD20, which is downregulated by antibody- secreting cells, these antibody therapies are thought to function by targeting precursors to these antibody- secreting B cells and/or B cells with antigen- presenting or other pathogenic functions.

## Bibliografia

1. Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. Nature. 2005; 435: 590-597.
2. Crotty S. Follicular helper CD4 T cells (TFH). Ann Rev Immunol. 2011; 29: 621-663.
3. Manzo A, Bombardieri M, Humby F, Pitzalis C. Secondary and ectopic lymphoid tissue responses in rheumatoid arthritis: from inflammation to autoimmunity and tissue damage/remodeling. Immunol Rev. 2010; 233: 267-285.
4. Linterman MA, Rigby RJ, Wong RK, Yu D, Brink R, Cannons JL, et al. Follicular helper T cells are required for systemic autoimmunity. J Exp Med. 2009; 206: 561-576.
5. Crotty S. A brief history of T cell help to B cells. Nat Rev Immunol. 2015; 15: 185-189.
6. Kawabe T, Naka T, Yoshida K, Tanaka T, Fujiwara H, Suematsu S, et al. The

TORNA ALL'INDICE

immune responses in CD40-deficient mice: impaired immunoglobulin class switching and germinal center formation. Immunity. 1994; 1: 167-178.
7. Takahashi Y, Dutta PR, Cerasoli DM, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. V. Affinity maturation develops in two stages of clonal selection. J Exp Med. 1998 ;187: 885-895.
8. Ferguson SE, Han S, Kelsoe C, Thompson CB. CD28 is required for germinal center formation. J Immunol. 1996; 156: 4576-4581.
9. Wang CJ, Heuts F, Ovcinnikovs V, Wardzinski L, Bowers C, Schmidt EM, et al. CTLA-4 controls follicular helper T-cell differentiation by regulating the strength of CD28 engagement. Proc Natl Acad Sci USA. 2015; 112: 524-529.
10. Wing JB, Ise W, Kurosaki T, Sakaguchi S. Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4. Immunity. 2014; 41: 1013-1025.
11. Walker LSK, Gulbranson-Judge A, Flynn S, Brocker T, Raykundalia C, Goodall M, et al. Compromised OX40 function in CD28-deficient mice is linked with failure to develop CXC chemokine receptor 5-positive CD4 cells and germinal centers. J Exp Med. 1999; 190: 1115-122.
12. Flynn S, Toellner K-M, Raykundalia C, Goodall M, Lane P. CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. J Exp Med. 1998; 188: 297-304.
13. Brocker T, Gulbranson-Judge A, Flynn S, Riedinger M, Raykundalia C, Lane P. CD4 T cell traffic control: in vivo evidence that ligation of OX40 on CD4 T cells by OX40-ligand expressed on dendritic cells leads to the accumulation of CD4 T cells in B follicles. Eur J Immunol. 1999; 29: 1610-1616.
14. Boettler T, Moeckel F, Cheng Y, Heeg M, Salek-Ardakani S, Crotty S, et al. OX40 facilitates control of a persistent virus infection. PLoS Pathog. 2012; 8: e1002913.
15. Tahiliani V, Hutchinson TE, Abboud G, Croft M, Salek-Ardakani S. OX40 cooperates with ICOS to amplify follicular Th cell development and germinal center reactions during infection. J Immunol. 2017; 198: 218-228.
16. McAdam AJ, Greenwald RJ, Levin MA, Chernova T, Malenkovich N, Ling V, et al. Icos is critical for CD40-mediated antibody class switching. Nature. 2001; 409: 102-105.
17. Choi YS, Kageyama R, Eto D, Escobar TC, Johnston RJ, Monticelli L, et al. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. Immunity. 2011; 34: 932-946.
18. Gigoux M, Shang J, Pak Y, Xu M, Choe J, Mak TW, et al. Inducible costimulator promotes helper T-cell differentiation through phosphoinositide 3-kinase. Proc Natl Acad Sci USA. 2009; 106: 20371-20376.
19. Yuseff MI, Pierobon P, Reversat A, Lennon-Dumenil AM. How B cells capture, process and present antigens: a crucial role for cell polarity. Nat Rev Immunol. 2013; 13: 475-486.

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20. Lane P, Traunecker A, Hubele S, Inui S, Lanzavecchia A, Gray D. Activated human T cells express a ligand for the human B cell-associated antigen CD40 which participates in T cell-dependent activation of B lymphocytes. Eur J Immunol. 1992; 22: 2573-2578.
21. Avalos AM, Meyer-Wentrup F, Ploegh HL. B-cell receptor signaling in lymphoid malignancies and autoimmunity. Adv Immunol. 2014; 123: 1-49.
22. Ng LG, Sutherland AP, Newton R, Qian F, Cachero TG, Scott ML, et al. B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells. J Immunol. 2004; 173: 807-817.
23. Dienz O, Eaton SM, Bond JP, Neveu W, Moquin D, Noubade R, et al. The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells. J Exp Med. 2009; 206: 69-78.
24. Liu SM, King C. IL-21-producing Th cells in immunity and autoimmunity. J Immunol. 2013; 191: 3501-3506.
25. Rydén-Aulin M, Boumpas D, Bultink I, Callejas Rubio JL, Caminal-Montero L, Castro A, et al. Off-label use of rituximab for systemic lupus erythematosus in Europe. Lupus Sci Med. 2016; 3: e000163.
26. DiLillo DJ, Hamaguchi Y, Ueda Y, Yang K, Uchida J, Haas KM, et al. Maintenance of long-lived plasma cells and serological memory despite mature and memory B cell depletion during CD20 immunotherapy in mice. J Immunol. 2008; 180: 361-371.
27. Mysler EF, Spindler AJ, Guzman R, Bijl M, Jayne D, Furie RA, Houssiau FA, et al. Efficacy and safety of ocrelizumab in active proliferative lupus nephritis: results from a randomized, double-blind, phase III study. Arthritis Rheum. 2013; 65: 2368-2379.

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# 10

# Immunology in clinical oncology: open questions

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Over the past 15 years, advances in the characterization of the molecular mechanisms of carcinogenesis, of anticancer immune response, and of tumor resistance/escape, allowed to identify several pathways that have become targets of specific anticancer agents. In particular, modulation of the innate anticancer immune response has become a key target for pharmacological interventions (1), and finally combinations of immunotherapeutics and more traditional anticancer therapies (e.g. chemotherapy, targeted agents, and radiation therapy) (2-4) are presently emerging as a possible new step forward in our long-lasting fight against cancer.

The development of all these new treatment strategies revolutionized the medical treatment of many different tumors, greatly improving the outcomes of many cancer patients. However, oncologists had also to face relevant challenges the use of these agents brought to their attention, including: how to evaluate response (5); how to interpret the results of clinical trials, and which measure to use for this purpose (at a certain extent, a consequence of the previous issue) (6); and, more practically, how to deal with novel, and sometimes ill-defined, toxicities (7).

Differently from cytotoxic chemotherapy, treatment with immune checkpoint inhibitors (but also with targeted agents) may not result in significant reductions in tumour size, and thus standard evaluation criteria based on serial tumour measurements proved to be inappropriate for evaluating response to therapy. In fact, these agents yield low traditional response rates, a type of antitumor activity that do not reflect the increased, and prolonged, disease control they often induce (8). Furthermore, because of the indirect mechanism of action of immune checkpoint inhibitors, which ultimately stimulate the immune system, atypical response patterns (9) such as delayed responses, transient enlargements of target lesions, or even appearance of new lesions, often before subsequent tumour shrinkage (i.e. pseudo-progressions) (10), are commonly observed at re-evaluation imaging studies during or even after treatment, not to take into account that dramatic phenomenon which is hyperprogression (11).

The complexity of choosing the right endpoint, and correctly interpreting study's results are direct consequences of the above issues. Traditionally, overall survival (OS) is the gold standard among efficacy endpoints in clinical trials, and



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median OS is therefore often used as the primary endpoint of interest. However, since median OS may be confounded by the sequential use of a number of active anticancer therapies, progression-free survival (PFS) is often used instead and considered a surrogate marker of OS, despite the fact that this proved to be true only in selected. However, a discrepancy between PFS and OS is also extremely common in the case of immune checkpoint inhibitors, mainly due to their latency of action.

For these reasons, the application in clinical trials of novel response assessment tools and of innovative statistical methodologies (e.g. milestone analysis, restricted mean survival time, parametric models, etc …), specifically designed for novel immunotherapeutics (12, 13), and aimed at adequately quantify the fraction of patients who are possibly cured, i.e. those represented in the tails of the survival curves, have been advocated.

Another huge issue is the identification of biomarkers of treatment efficacy. Porgrammed Death-1 (PD1) immunohistochemical expression has been advocated as the best way to predict who will eventually respond to immune checkpoint inhibitors, or else derive no benefit at all from these agents. What is now clear, with few exceptions, is that PD1 expression has prognostic, but not predictive value, not to take into account other unsolved question, e.g. how to measure it, where to check its expression, which is the ideal cut-off, and so on (14-16).

Finally, another clinically relevant task Oncologists are asked to deal with (which however is best handled through a multidisciplinary approach), is the management of treatment-related adverse events (AEs).

Over the years, like opening Pandoras's box (17), a wide array of previously unrecognised and ill-defined AEs of these novel drugs have been increasingly observed, which we had to learn how to deal with (18). Although official guidelines do not exist for many of the AEs caused by targeted agents, for immune-related AEs (irAEs) caused by immune checkpoint inhibitors specific guidelines have been recently developed (19).

What is clear is that the daily work of medical oncologists is rapidly changing and that new challenges are emerging, which needs highly specialized and trained specialist. The years of "one size (one physician) fits all" (every cancer patients) is at its dawn, irrespective of what Politicians are often telling us.

## Bibliografia

1. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011; 480: 480-489.
2. Gotwals P, Cameron S, Cipolletta D, et al. Prospects for combining targeted and conventional cancer therapy with immunotherapy. Nat Rev Cancer. 2017; 17: 286-301.
3. Eckert F, Zwirner K, Boeke S, Thorwarth D, Zips D, Huber SM. Rationale for Combining Radiotherapy and Immune Checkpoint Inhibition for Patients With Hypoxic Tumors. Front Immunol. 2019; 10: 407.
4. Porta C, Rizzo M. Immune-based combination therapy for metastatic kidney

TORNA ALL'INDICE

cancer. Nat Rev Nephrol 2019 Apr 16. doi: 10.1038/s41581-019-0149-0. [Epub ahead of print].
5. Nishino M, Ramaiya NH, Hatabu H, Hodi FS. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. Nat Rev Clin Oncol. 2017; 14: 655-668.
6. Golan T, Milella M, Ackerstein A, Berger R. The changing face of clinical trials in the personalized medicine and immuno-oncology era: report from the international congress on clinical trials in Oncology & Hemato-Oncology (ICTO 2017). J Exp Clin Cancer Res. 2017; 36: 192.
7. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016; 54: 139-148.
8. León L, García-Figueiras R, Suárez C, et al. Recommendations for the clinical and radiological evaluation of response to treatment in metastatic renal cell cancer. Target Oncol. 2014; 9: 9-24.
9. Queirolo P, Spagnolo F. Atypical responses in patients with advanced melanoma, lung cancer, renal-cell carcinoma and other solid tumors treated with anti-PD-1 drugs: A systematic review. Cancer Treat Rev. 2017; 59: 71-78.
10. Borcoman E, Nandikolla A, Long G, Goel S, Le Tourneau C. Patterns of Response and Progression to Immunotherapy. Am Soc Clin Oncol Educ Book. 2018; 38: 169-178.
11. Kurman JS, Murgu SD. Hyperprogressive disease in patients with non-small cell lung cancer on immunotherapy. J Thorac Dis. 2018; 10: 1124-1128.
12. Ferrara R, Pilotto S, Caccese M, et al. Do immune checkpoint inhibitors need new studies methodology? J Thorac Dis. 2018; 10 (Suppl. 13): S1564-80.
13. Pak K, No H, Kin DH, et al. Interpretability of cancer clinical trial results using restricted mean survival time as an alternative to hazard ratio. JAMA Oncol. 2017; 3: 1692-1696.
14. Ilie M, Hofman V, Dietel M, Soria JC, Hofman P. Assessment of the PD-L1 status by immunohistochemistry: challenges and perspectives for therapeutic strategies in lung cancer patients. Virchows Arch. 2016; 468: 511-525.
15. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol. 2016; 17: e542-551.
16. Teng F, Meng X, Kong L, Yu J. Progress and challenges of predictive biomarkers of anti PD-1/PD-L1 immunotherapy: A systematic review. Cancer Lett. 2018; 414: 166-173.
17. Porta C, Paglino C, Imarisio I, Bonomi L. Uncovering Pandora's vase: the growing problem of new toxicities from novel anticancer agents. The case of sorafenib and sunitinib. Clin Exp Med. 2007; 7: 127-134.
18. Champiat S, Lambotte O, Barreau E, et al. Management of immune checkpoint blockade dysimmune toxicities: a collaborative position paper. Ann Oncol. 2016; 27: 559-574.
19. Haanen JBAG, Carbonnel F, Robert C, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017; 28 (Suppl. 4): iv119-42.

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# 11

# Immune-related adverse events from cancer immunotherapy with check-points inhibitors

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Immunotherapy has revolutionized the treatment of cancer and is now considered central to the clinical management (1). The immunotherapy field is evolving rapidly, and although a variety of treatment modalities exist, including breakthroughs in cellular therapies, the most commonly used approach is to administer monoclonal antibodies that are specific for regulatory checkpoint molecules, that is, checkpoint inhibitors (CPIs). CPIs regulate T cell activation and effector function and are highly effective in treating a wide range of cancers. Attendant to the use of these therapies has been a shift of patient toxicity profiles away from immunosuppressive complications, especially serious infections associated with traditional chemotherapies, to a new spectrum of adverse events of autoimmune or autoinflammatory origin, often referred to as immune-related adverse events (irAEs) (2). irAEs can occur as single toxicities or in combination and seem to develop via a process of immune activation that is not entirely understood. Remarkably, irAEs have been reported to occur in almost every organ system (2). Among these adverse events are rheumatic complications (3-7) that not only are challenging to diagnose and treat but also seem to be nosologically distinct from other irAEs.

irAEs can affect almost any organ system and are remarkably common in patients treated with CPIs. In some clinical trials, up to 90% of patients experienced irAEs of any grade of toxicity (8), and in a meta-analysis, it was estimated to be closer to 75% with anti-CTLA4 and 30% with anti-PD-1 and/or anti-PD-L1 therapies (9). A more informative appraisal of the extent of irAEs can be made with reference to the grading or severity of the toxicity itself. irAEs are graded according to the Common Terminology Criteria for Adverse Events (CTCAE), in which grade 1 and grade 2 are considered mild, grade 3 and grade 4 are considered severe, and grade 5 is fatal (10).

Although dermatological, gastrointestinal and endocrine immune-related adverse events (irAEs) are the most frequently reported irAEs, irAEs can affect nearly every organ system and can range from mild and self-limiting to severe and life threatening. Many of these irAEs mirror rheumatic diseases.

The literature on rheumatic irAEs is growing rapidly and de novo clinical syndromes, many of which are phenotypically similar to classic rheumatic diseases, have been reported to be a potential consequence of cancer immunotherapy. Nearly every major category of rheumatic disease, including inflammatory

arthritis, myositis, vasculitis and scleroderma, is mirrored by a category of irAEs resulting from CPIs.

The incidence of rheumatic irAEs is less well characterized than the incidence of other irAEs, such as colitis, pneumonitis and thyroid disease. Some information about the incidence of rheumatic irAEs can be found in reports from observational studies. One single-centre study showed a 5.1% (10 of 195 patients) incidence of inflammatory arthritis in patients treated with a PD-1 inhibitor with or without ipilimumab for metastasized cutaneous malignancies (11). Other studies reported similar incidence of arthritis (12, 13). Myositis is less common than inflammatory arthritis but seems to be on the rise; in 1 retrospective study of 654 patients treated with PD-1 inhibitors, 5 patients developed biopsy-proven myositis (0.8%) (14). Future prospective studies with active ascertainment of rheumatic irAEs are critical to better understand the burden of these de novo rheumatic diseases.

Inflammatory arthritis resulting from CPIs has been described in several different case series and retrospective cohort studies (14, 15, 16); both small and large joints were involved, and instances of oligoarthritis and polyarthritis (with polyarthritis being more common) were recorded. The severity of the arthritis can range widely from mild, requiring only NSAIDs or low-dose prednisone, to severe, requiring treatment with TNF inhibitors (14) or IL-6 receptor inhibitors. Time to onset ranges from immediately after a single therapeutic dose to 2 years after starting therapy. Although most patients with inflammatory arthritis are seronegative for rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPAs), a subgroup that is seropositive for RF and/or ACPAs has been reported (16). In general, in patients with inflammatory arthritis, imaging with MRI and musculoskeletal ultrasonography has shown erosive disease, tenosynovitis, Doppler-positive synovitis and joint effusions.

Isolated polymyalgia rheumatica and giant cell arteritis (GCA) with features of polymyalgia rheumatica have been reported after treatment with both anti-CTLA4 and anti-PD-1 CPIs, but polymyalgia rheumatica symptoms are more common than the symptoms of GCA that have been primarily described in case reports. Symptoms mirror those of the traditional forms of disease for both entities, including hip and shoulder girdle stiffness, temporal headache, jaw claudication and one incidence of amaurosis fugax. In temporal artery biopsy samples, arteritis, disruption of the elastic lamina, and intimal proliferation have been detected (17).

Cases of new onset myositis, mostly consistent with polymyositis, have been seen in CPI-treated patients whereas dermatomyositis is rarely reported (18, 19). Creatine kinase tends to be increased by a factor of ten or more, and proximal muscles are typically affected.

Sicca syndrome, (dry mouth with or without dry eyes), can occur in patients treated with CPIs. These symptoms can have an acute onset and a substantial detrimental effect on patient quality of life. In contrast to patients with the classical form of Sicca syndrome, most of these patients do not have anti-Ro or anti-La antibodies or concomitant parotitis, but in individual patients, these features can occur (3, 20).

Two cases of systemic sclerosis have been reported in patients with metastatic melanoma treated with pembrolizumab (anti-PD-1), one with diffuse and one with limited skin involvement (21).

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Other types of vasculitis have been reported in isolated case reports, including single organ vasculitis involving the central nervous system, uterus and retina. In addition, a single case of granulomatosis with polyangiitis with lung and renal involvement occurring in a patient treated with ipilimumab followed by pembrolizumab therapy has been reported (22).

## Treatment

It is of outmost importance to treat rheumatic irAEs with an inter-professional team of clinicians to enable optimal therapy of an underlying cancer. The specific management for rheumatic irAEs is dependent on the severity of the event, the organ systems involved and therapies that are known to be effective in treating the related rheumatic disease. Plans for further CPI therapy or other cancer therapy (for example, chemotherapy) are also important aspects of management of these irAEs. Rheumatologists must therefore work closely with oncologists, and early referral to rheumatology clinics for suspected rheumatic irAEs is important to facilitate early diagnosis and treatment.

The general framework for treatment of irAEs (on the basis of severity) has been outlined by a multidisciplinary working group, and subsequent guidelines from the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology have been published for management of many irAEs (10, 23). For grade 1 events, systemic corticosteroids are generally not needed and immunotherapy is continued. For grade 2 or higher events, corticosteroids and other immunomodulatory agents can be indicated, depending on the type of event. For grade 3 or higher events, immunotherapy is typically held or discontinued. Specific recommendations for treatment for inflammatory arthritis, myositis and polymyalgia-like syndrome have been proposed by the ASCO guidelines25 but not for other rheumatic irAEs.

*Pre-existing rheumatic diseases*

One of the most intriguing and still unresolved clinical problem is represented by patients with pre-existing rheumatic or other autoimmune diseases who develop cancer, and in particular if CPI treatment is a safe option in these cases. Unfortunately, such patients were not included in the clinical trials that led to FDA/EMA approval of these immunotherapies. The results of several small retrospective studies and a meta-analysis indicate that up to 50% of patients with immune-mediated inflammatory diseases who are treated with CPIs will experience disease flares, and another 20-30% might develop de novo irAEs while receiving CPI therapy, yet most of these irAEs can be managed, and many patients benefit from CPI therapy (24). Also, in a small prospective study of a French registry, CPI-treated patients with pre-existing autoimmunity were both more likely to have new onset irAEs and to have sooner onset of such complications than those without pre-existing autoimmunity (25). Other unanswered questions include whether patients experiencing chronic rheumatic irAEs, once controlled with DMARDs, can then be effectively treated for cancer if needed.

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In the absence of clear data coming from the literature, it should be underlined that rheumatologists should have a central role in the diagnosis and management of irAEs (26). The presence of multiple irAEs in any given patient requires coordination among specialists and also often requires selection of immunosuppressive drugs that may affect multiple organ systems. With the rapid proliferation and therapeutic application of various CPI-based cancer immunotherapies, practising rheumatologists are needed urgently to increase their knowledge regarding diagnosis and management of irAEs; therefore, educational initiatives are also recommended. Rheumatologists must stay informed of this new area of rheumatic diseases and become central partners in inter-professional teams engaged in the management of irAEs and the research that is essential to increase our understanding of them.

**Bibliografia**

1. Hoos A. Development of immuno-oncology drugs - from CTLA4 to PD1 to the next generations. Nat Rev Drug Discov. 2016; 15: 235-247.
2. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med. 2018; 378: 158-168.
3. Cappelli LC, Gutierrez AK, Bingham CO. 3rd, Shah, A. A. Rheumatic and musculoskeletal immune-related adverse events due to immune checkpoint inhibitors: a systematic review of the literature. Arthritis Care Res. 2017; 69: 1751-1763.
4. Suarez-Almazor M E, Kim ST, Abdel-Wahab N, Diab A. Review: immune-related adverse events with use of checkpoint inhibitors for immunotherapy of cancer. Arthritis Rheumatol. 2017; 69: 687-699.
5. The Lancet Oncology. Calling time on the immunotherapy gold rush. Lancet Oncol. 2017; 18: 981.
6. van der Vlist M, Kuball J, Radstake TR, Meyaard L. Immune checkpoints and rheumatic diseases: what can cancer immunotherapy teach us? Nat Rev Rheumatol 2016; 12: 593-604.
7. Calabrese LH, Calabrese C, Cappelli LC. Rheumatic immune-related adverse events from cancer immunotherapy. Nat Rev Rheumatol. 2018; 14: 569-579.
8. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, et al. Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy. N Engl J Med. 2016; 375, 1845-1855.
9. Bertrand A, Kostine M, Barnetche T, Truchetet ME, Schaeverbeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. BMC Med. 2015; 13: 211.
10. Puzanov I, et al. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. J Immunother Cancer. 2017; 5: 95.
11. Buder-Bakhaya K, et al. Characterization of arthralgia induced by PD-1 antibody treatment in patients with metastasized cutaneous malignancies. Cancer Immunol Immunother. 2018; 67: 175-182.

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12. Lidar M, et al. Rheumatic manifestations among cancer patients treated with immune checkpoint inhibitors. Autoimmun Rev. 2018; 17: 284-289.
13. Le Burel S, et al. Prevalence of immune-related systemic adverse events in patients treated with anti-programmed cell death 1/anti-programmed cell death-ligand 1 agents: a single-centre pharmacovigilance database analysis. Eur J Cancer. 2017; 82: 34-44.
14. Liewluck T, Kao JC, Mauermann ML. PD-1 inhibitor-associated myopathies: emerging immune-mediated myopathies. J Immunother. 2017; 41: 208-211.
15. Smith MH, Bass AR. Arthritis after cancer immunotherapy: symptom duration and treatment response. Arthritis Care Res. 2019; 71: 362-366.
16. Belkhir R, et al. Rheumatoid arthritis and polymyalgia rheumatica occurring after immune checkpoint inhibitor treatment. Ann Rheum Dis. 2017; 76: 1747-1750.
17. Goldstein BL, Gedmintas L, Todd DJ. Drug-associated polymyalgia rheumatica/giant cell arteritis occurring in two patients after treatment with ipilimumab, an antagonist of ctla-4. Arthritis Rheumatol. 2014; 66: 768-769.
18. Sheik Ali S, et al. Drug-associated dermatomyositis following ipilimumab therapy: a novel immune-mediated adverse event associated with cytotoxic T-lymphocyte antigen 4 blockade. JAMA Dermatol. 2015; 151: 195-199.
19. Daoussis D, Kraniotis P, Liossis SN, Solomou A. Immune checkpoint inhibitor-induced myo-fasciitis. Rheumatology. 2017; 56: 2161.
20. Cappelli LC, et al. Inflammatory arthritis and sicca syndrome induced by nivolumab and ipilimumab. Ann Rheum Dis. 2017; 76: 43-50.
21. Barbosa NS, et al. Scleroderma induced by pembrolizumab: a case series. Mayo Clin. Proc. 2017; 92: 1158-1163.
22. Daxini A, Cronin K, Sreih AG. Vasculitis associated with immune checkpoint inhibitors-a systematic review. Clin. Rheumatol. 2018; 37: 2579-2584.
23. Haanen J, et al. Management of toxicities from immunotherapy: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018; 28: iv119-iv142.
24. Abdel-Wahab N, Alshawa A, Suarez-Almazor ME. Adverse events in cancer immunotherapy. Adv. Exp. Med. Biol. 2017; 995: 155-174.
25. Danlos FX, et al. Safety and efficacy of anti-programmed death 1 antibodies in patients with cancer and pre-existing autoimmune or inflammatory disease. Eur J Cancer. 2018; 91: 21-29.
26. Calabrese L, Mariette X. The evolving role of the rheumatologist in the management of immune-related adverse events (irAEs) caused by cancer immunotherapy. Ann Rheum Dis. 2018; 77: 162-164.

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# 12

# Immune checkpoint inhibitors in cancer therapies

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Several decades ago It has been hypothesized that a process known as *tumor immunosurveillance*, consisting in the immune defences that recognize cancer cells, would exist in mammals to contrast cancer development. Tumor immune surveillance is based on the ability of the immune system to detect and destroy cancer cells through the same pathways used to protect us from pathogens. A more recent view acknowledges that the in vivo process of neoplastic transformation generates a complex network of immune responses, whose final outcome strongly depends on intrinsic tumor features. Indeed, tumor immunity is a systemic process regulated by the dynamic interplay of *immunoactivating anti-tumor* and *immunosuppressive protumor* responses.

Based on these two contrasting forces, tumors can be dichomotized into *hot vs cold* cancers, where the first are tumors with prevailing antitumor immunity and the latter are lesions with predominant immunosuppressive response. *Hot* tumors are featured by the expression of antigenic determinants that primes specific T lymphocytes to infiltrate tumor site. The antigenic repertoire of these neoplasms is the result of the alterations in the tumor protein content (due to cancer-related genetic mutations, deletions, translocations, epigenetic regulations, ectopic or quantitatively altered expressions, viral etiology) and the ability of the tumor cell to process and present the altered proteins to T lymphocytes. Activated anti-tumor T cells are primed against tumor antigens within the regional lymph nodes, migrate to the tumor and then infiltrate the lesion to contrast cancer cell growth and expansion. The positive prognostic impact that T cell infiltrate plays in most human cancers demostrates the role of T lymphcytes in cancer immune surveillance.

However, the immune system has developed to act in acute conditions, by rapidly mediating antigen recognition, clonal expansion and pathogen elimination. Afterwards, to avoid the damage of sorrounding normal cells and maintain tissue homeostasis, T cells are programmed to undergo a process known as “clonal contraction”, finalized to block the immune response by neutralizing most activated T cells. Among the large array of redundant mechanisms devoted to this process, the most relevant pathway is represented by the expression of immune checkpoints. These receptors, including the nowdays well-known PD-1/PD-L1 and CTLA4 axes (and other molcules such as LAG3 and TIM3), are substantial-

ly responsible to mitigate T cell activation and functions. Indeed, infiltrating T cells within tumor microenvironment are featured by the high expression of such molecules, which explains the block in proliferation, the reduced secretion of cytokines and the limited expression of cytolytic mediators that is usually here detected. The high expression of immune checkpoints by lymphocytes of *hot* tumors indicates that these cells, albeit efficiently triggered by tumor antigenicity, are most intristically unable to eliminate the tumor in a complete fashion because of the physicological mechanisms regulating their activity. This evidence explains why the administration of immune checkpoint inhibitors (representing the major therapeutic tool recently entering clinical practise in the context of cancer immunotherapy) mediates the reinvigoration of anti-tumor T cell reactivity and the onset of immuno-mediated tumor control in patients with pre-existing immunity. Intense research has been ongoing over the last decade to identify biomarkers reflecting the level of pre-existing anti-tumor immunity, in order to select patients responding to immune checkpoint inhibitors. So far, several candidates are available and still under prospective validation.

The other key biomarker needing to be identified is that able to define *cold* tumors and the mechanisms underlying their intrinsic resistantance to immune checkpoint inhibitors. *Cold* tumors, lacking spontaneous T cell infiltrate, are instead usually featured by the presence of stroma and immune cells associated with chronic inflammation (*Figure 1*). Preclinical data proved that in *cold* tum-

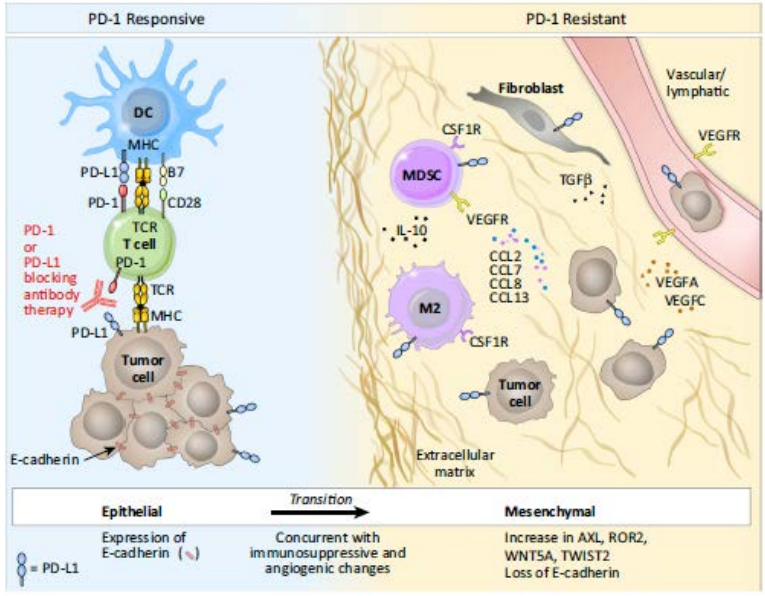


***Fig. 1*** - Dichotomized view of immune tumor microenviroment, with HOT tumors infiltrated by T cells and responding to immunotherapy (left) and COLD tumors displaying a prevaling myeloid immunosuppressive and proangiogenic microenvironment, usually showing resistance to immuntherapy (Bu et al., Trends Molecular Medicine 2016).

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ors, specific oncogenic pathways drive the secretion of soluble factors capable of accruing immunosuppressive elements at tumor site. Multiple molecular mechanisms are emerging that might explain the inability of T cells to infiltrate cold tumors, most of which are related to the capacity of tumor cells to create an hostile mileu hardly accessible to T cells. In terms of clinical strategies, new drugs are under development to turn *cold tumors into hot* by targeting the immunosuppressive microevironment or generating stronger antitumor T cells. However, a common pattern that characterizes *cold* tumors is the ability to modify myelopoiesis in the bone marrow and cause the mobilization of immature myeloid cells. These cells are then accrued to tumor site where they sustain tumor growth by suppressing anti-tumor immunity and promoting neangiogenesis, stroma remodelling and metastatization. Removal of myeloid cells in tumor-bearing mice restores sensitivity to immune checkpoint inhibitors in different tumor models. Many stardard cancer therapies can interfere with myelopoiesis and reduce the immunosuppressive pressure of myeloid cells, starting from chemotherapy to antiangiogenics and defined tyrosine-kinase inhibitors. This explains why the association of these drugs with immune checkpoint blockers increases clinical benefit in multiple tumor histotypes. Understanding the mechanisms of resistance represents the new frontier for allowing host immunity to become an efficient anti-cancer therapy in standard clinical practise.

**Bibliografia**

1. Finn OJ. A Believer's Overview of Cancer Immunosurveillance and Immunotherapy. J Immunol. 2018; 200: 385-391.
2. Li J, Byrne KT, Yan F, et al. Tumor cell-intrinsic factors underlie heterogeneity of immune cell infiltration and response to immunotherapy. Immunity. 2018; 49: 178-193.
3. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nature Reviews Drug Discovery. 2019; 18: 197-218.
4. https://www.sciencedirect.com/topics/medicine-and-dentistry/tumor-immunogenicity
5. Finn OJ. Human Tumor Antigens Yesterday, Today, and Tomorrow. Cancer Immunol Res. 2017; 5: 347-354.
6. Barnes AT, Amir E. HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer Br J Cancer. 2017; 117: 45-460.
7. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018; 359: 1350-1355.
8. Sharpe AH, Pauken KE.The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol. 2018; 18: 153-167.
9. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer. 2019; 19: 133-150.
10. Spencer C. Wei, Colm R. Duffy and James P. Allison. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy, Cancer Discov. 2018; 8, 9: 1069-1086.

TORNA ALL'INDICE

11. Park YJ, Kuen DS Chung Y. Future prospects of immune checkpoint blockade in cancer: from response prediction to overcoming resistance. Experimental & Molecular Medicine. 2018; 50: 109.
12. Jonathan J, Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nature Reviews Cancer. 2019; 19: 133-150.
13. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer. 2019; 19: 133-150.
14. Xia Bu Xoney KM, and Freeman GJ. Learning from PD-1 Resistance: New Combination Strategies, Trends in Molecular Medicine. 2016; 22: 448-451.
15. Talmadge JE, , Mah Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer. 2013; 13: 739-752.
16. Fleming V, Hu X, Weber R, Nagibin V, Groth C, Altevogt P, Utikal J, Umansky V. Targeting Myeloid-Derived Suppressor Cells to Bypass Tumor-Induced Immunosuppression. Front Immunol. 2018; 9: 398.
17. Wellenstein MD, de Visser KE Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. Immunity. 2018; 48: 399-416.

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# 13

# Regolazione trascrizionale e post-transcrizionale dei linfociti T umani

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T helper (Th) lymphocytes have a central role in orchestrating immune responses to an invading pathogen through their ability to produce high levels of effector cytokines. Following the first recognition of a foreign infectious or noxious agent, antigen inexperienced naïve Th cells undergo rapid proliferation and at the same time differentiate into an array of memory, effector and regulatory subsets, with responses tailored towards the specific pathogen being recognized. Specifically, activated naïve Th cells differentiate into a variety of subsets commonly defined by the cytokines produced, the transcription factors expressed and the type of protective response provided. For example, Th1 cells produce IFN-γ, express the transcription factor T-BET, and are primarily involved in the response to intracellular pathogens, while Th17 cells produce IL-17, express the transcription factor RORC and have an important role in the protection against fungi (1). Th lymphocytes can also differentiate into regulatory T (Treg) cells, expressing the transcription factor FOXP3 and crucial in regulating immune responses to avoid excessive reactions and damage to the host (1). T cell differentiation occurs upon T cell receptor (TCR) stimulation and in the context of an extracellular milieu of specific cytokines determined by the pathogenic stimuli. The relationship between the various Th subsets is complex and regulated by a delicate equilibrium between phenotypic stability and plasticity, namely the ability of a given cell to stably 'remember' a phenotype characterized by a specific cytokine-producing profile *vs.* the ability to rapidly adapt to the changing environmental conditions. At the molecular level, such processes are mediated by and are regulated through the combinatorial action of transcription factors, signaling molecules, epigenetic regulators, as well as post-transcriptional mechanisms, to finally establish transcriptomes distinctive of each subset (2, 3). Understanding the mechanisms that modulate Th lymphocyte functions is crucial to decipher normal and pathogenic immune responses in humans.

Here, I will provide some examples of different layers of regulation of gene expression in human T lymphocytes, such as DNA methylation, transcription factors and post-transcriptional regulation mediated by microRNAs (miRNAs) and RNA-binding proteins (RBPs).

Among the epigenetic regulators, the methylation of the cytosine base (5mC) in the genomic DNA is essential for mammalian development and for cell lineage



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specification, and is intimately linked with the regulation of gene expression (4). The bulk genomic methylation patterns are mostly static in differentiated cells and tissues, with large stably methylated regions including the inactive X chromosome, imprinted genes, pericentromeric repeats and other repeated elements, and transposable elements (5).

Despite its stability and heritability across cell division, dynamic changes in 5mC deposition are observed during development and differentiation, and are deemed to be necessary for the establishment of stable cell-specific gene expression programs (3). Once deposited in the genome, 5mC can be removed either through passive dilution during DNA replication, which occurs if the methyl mark is not copied on the nascent DNA strand, or through active mechanisms mediated by enzymes of the TET (Ten-eleven translocation) family. The 5mC mark is oxidized by TET proteins to 5-hydroxymethylcytosine (5hmC), which can then undergo further oxidation processes (6, 7). 5hmC is however a stable mark that can accumulate to significant levels, contributing to the regulation of gene expression, possibly by recruiting readers of this modification. The 5hmC modification can therefore act both as an intermediate of active DNA demethylation and as a stable epigenetic mark (3).

The importance of post-transcriptional regulation of mRNA expression in the modulation of immune responses is also becoming increasingly clear. Such regulatory mechanisms include miRNAs as well as an assortment of RBPs that regulate mRNA polyadenylation, splicing, stability, nuclear export and translation. Mechanisms of post-transcriptional regulation of gene expression are particularly important for immune cells, in which the expression of cytokines has to change rapidly in response to danger signals, but it also must be quickly turned off to avoid excessive inflammation and tissue damage. Indeed, many mRNAs encoding for cytokines have long 3'-untranslated regions (UTRs) containing many *cis*-elements that enable interactions with *trans*-acting factors to render these mRNAs unstable and/ or kept in a translationally silent state until needed (8). Such *trans*-acting factors may include the miRISC (miRNA-containing RNA-induced silencing complex) recognizing sequences with partial complementarity to miRNAs, or RBPs interacting with specific stem-loop structures or linear sequence motifs in mRNAs. For instance, several miRNAs were shown to impact human T cell biology (2, 9, 10), while RBPs such as the Regnase family of proteins was shown to strongly impact immune and inflammatory responses. These proteins are zinc finger CCCH-type- containing RNases acting as negative regulators of inflammation (11-13) through the degradation and turnover of many mRNAs involved in inflammation (12, 14-17). Specifically, deficiency of *Zc3h12a* (encoding for Regnase-1) in mice led to an autoimmune-like phenotype with T cell hyperactivation (12, 18), while deficiency of *Zc3h12d* (encoding for Regnase-4) led to increased stability of cytokine mRNAs, increased levels of IL-17A and persistent severe paralysis in models of experimental autoimmune encephalomyelitis (EAE), highlighting a role in modulating the pro-inflammatory, potentially pathogenic phenotype of T lymphocytes, especially in the resolution phase of inflammation (19).

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**Bibliografia**


1. Kanno Y, Vahedi G, Hirahara K, Singleton K, O'Shea JJ. Transcriptional and epigenetic control of T helper cell specification: molecular mechanisms underlying commitment and plasticity. Annu Rev Immunol. 2012; 30: 707-731.
2. Monticelli S. MicroRNAs in T helper cell differentiation and plasticity. Semin Immunol. 2013; 25: 291-298.
3. Monticelli S. 2019. DNA (Hydroxy)Methylation in T Helper Lymphocytes. Trends Biochem Sci.
4. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nature reviews. Genetics. 2013; 14: 204-220.
5. Bird A. DNA methylation patterns and epigenetic memory. Genes & development. 2002; 16: 6-21.
6. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009; 324: 930-935.
7. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature. 2010; 466: 1129-1133.
8. Turner M, Galloway A, Vigorito E. Noncoding RNA and its associated proteins as regulatory elements of the immune system. Nat Immunol. 2014; 15: 484-491.
9. Mele F, Basso C, Leoni C, Aschenbrenner D, Becattini S, Latorre D, Lanzavecchia A, Sallusto F, Monticelli S. ERK phosphorylation and miR-181a expression modulate activation of human memory TH17 cells. Nat Commun. 2015; 6: 6431.
10. Rusca N, Deho L, Montagner S, Zielinski CE, Sica A, Sallusto F, Monticelli S. MiR-146a and NF-kappaB1 regulate mast cell survival and T lymphocyte differentiation. Mol Cell Biol. 2012; 32: 4432-4444.
11. Fu M, Blackshear PJ. RNA-binding proteins in immune regulation: a focus on CCCH zinc finger proteins. Nat Rev Immunol. 2017; 17: 130-143.
12. Uehata T, Iwasaki H, Vandenbon A, Matsushita K, Hernandez-Cuellar E, Kuniyoshi K, Satoh T, Mino T, Suzuki Y, Standley DM, Tsujimura T, Rakugi H, Isaka Y, Takeuchi O, Akira S. Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. Cell. 2013; 153: 1036-1049.
13. Matsushita K, Takeuchi O, Standley DM, Kumagai Y, Kawagoe T, Miyake T, Satoh T, Kato H, Tsujimura T, Nakamura H, Akira S. Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay. Nature. 2009; 458: 1185-1190.
14. Liang, J, Saad Y, Lei T, Wang J, Qi D, Yang Q, Kolattukudy PE, Fu M. MCP-induced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF- kappaB signaling. J Exp Med. 2010; 207: 2959-2973.
15. Huang S, Qi D, Liang J, Miao R, Minagawa K, Quinn T, Matsui T, Fan D, Liu J, Fu M. The putative tumor suppressor Zc3h12d modulates toll-like receptor signaling in macrophages. Cell Signal. 2012; 24: 569-576.


TORNA ALL'INDICE

16. Wawro M, Kochan J, Krzanik S, Jura J, Kasza A. Intact NYN/PIN-Like Domain is Crucial for the Degradation of Inflammation-Related Transcripts by ZC3H12D. J Cell Biochem. 2017; 118: 487-498.
17. Zhang H, Wang WC, Chen JK, Zhou L, Wang M, Wang ZD, Yang B, Xia YM, Lei S, Fu EQ, Jiang T. ZC3H12D attenuated inflammation responses by reducing mRNA stability of proinflammatory genes. Mol Immunol. 2015; 67: 206-212.
18. Cui X, Mino T, Yoshinaga M, Nakatsuka Y, Hia F, Yamasoba D, Tsujimura T, Tomonaga K, Suzuki Y, Uehata T, Takeuchi O. Regnase-1 and Roquin Nonredundantly Regulate Th1 Differentiation Causing Cardiac Inflammation and Fibrosis. J Immunol. 2017; 199: 4066-4077.
19. Minagawa K, Wakahashi K, Kawano H, Nishikawa S, Fukui C, Kawano Y, Asada N, Sato M, Sada A, Katayama Y, Matsui T. Posttranscriptional modulation of cytokine production in T cells for the regulation of excessive inflammation by TFL. J Immunol. 2014; 192: 1512-1524.

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# 14

# Terapia anticorpale in mieloma multiplo (MM) umano

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La terapia con anticorpi monoclonali (mAb) è partita anni fa con l'indicazione che potevano costituire un "proiettile magico". Il semplice trasferimento in vivo di una tecnica che genera anticorpi murini si è rivelata non priva di problemi. È stato necessario giungere a modificazioni della struttura dell'anticorpo attraverso tappe di umanizzazione o di generazione di molecole *full human*.

Nel contempo sono emerse altre evidenze a carico dell'azione degli anticorpi inizialmente considerate come molecole semplicemente leganti il bersaglio. Si è visto che alcuni specifici per molecole espresse sulla superficie di cellule sono in grado di indurre un suo blocco funzionale, altri invece erano in grado di innescare un'azione agonistica. Questo significa che l'anticorpo reagisce con un dominio della molecola bersaglio destinato ad ospitare un ligando naturale.

Ora è noto che tra gli anticorpi approvati per terapia alcuni hanno attività bloccante, altri sono inerti, altri ancora sono invece sinergistici (1).

CD38 è una molecola di superficie di tipo 2 (termine COOH posto all'interno della cellula) che si trova sulla superficie di leucociti normali: la sua espressione cresce: durante l'attivazione e durante la trasformazione tumorale. Le caratteristiche della molecola sono espresse in dettaglio in (2). In patologia umana la molecola CD38 ha trovato applicazioni nello studio della leucemia linfatica cronica B (B-CLL) e nel mieloma.

La B-CLL è la leucemia più diffusa nel mondo occidentale e presenta forme a differente gravità clinica. Una significativa frazione di queste leucemie esprime la molecola CD38: si è osservato che la frazione $CD38^+$ di B-CLL sono generalmente caratterizzate da una prognosi più grave. La molecola CD38 è stata ritenuta esercitare un ruolo non solo di marcatore, ma di contribuire alla migrazione di tali cellule al di fuori del sangue, fino a raggiungere linfonodi e midollo, ove sono al riparo dall'azione di farmaci (3).

Più complessa l'azione di CD38 nel mieloma multiplo, ove è stato scelto come bersaglio terapeutico.

Sono stati prodotti almeno 3 differenti anticorpi monoclonali allo scopo di ottenere eliminazione delle plasmacellule tumorali. Una di queste (Daratumumab) ha avuto l'approvazione della FDA, poi dell'agenzia europea ed infine di quella italiana. I risultati ottenuti sono stati descritti nella referenza (4). Un altro reagente con la stessa specificità è in corso di approvazione (5).



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La terapia con anticorpi anti-CD38 ha avuto importanti successi clinici, quadruplicando la sopravvivenza dei pazienti. Dal punto di vista generale sono invece emersi altri dati di interesse generale. In una visione schematica appare che lo stesso anticorpo è in grado di indurre lisi cellulare sul tumore mediata da ADCC, CDC e apoptosi. Tuttavia lo stesso anticorpo che reagisce con la stessa molecola ma espressa da effetti cellulari è in grado di indurre attivazione di effettori T e repressione di T regolatori.

Un quadro comprensivo potrebbe venire considerando le modalità con cui l'anticorpo reagisce con la molecola: smontando l'interazione anticorpo-recettori e la regione Fc delle globuline (FcR), è emerso che l'anticorpo terapeutico presentato in forma insolubile (cioè veicolato da cellule esprimenti FcR) porta ad una ridistribuzione della molecola bersaglio, che si accumula in patch di membrane (microvesicles) (6).

Un altro ruolo attribuito a CD38 è quello di essere un ectoenzima, in grado di regolare messaggi citoplasmatici coinvolti nella regolazione del calcio. Il mieloma ha strategie di immuno evasione basate sulla acidificazione dell'ambiente in cui cresce il mieloma. In queste condizioni, CD38 usa anche come substrato $NAD^+$ con produzione finale di adenosina (ADO), un potente immunosoppressore (7). Solitamente ADO viene ottenuta dal metabolismo di ATP.

Rimane ora da vedere se l'interferenza con questo percorso *in vivo* ha una valenza terapeutica.

Infine, difficile da spiegare che lo stesso anticorpo contro la stessa molecola è in grado di lanciare segnali che sembrano andare in direzioni opposte. Una risposta a questo potrebbe essere costituita dalla diversa densità sulla membrana cellulare. Più complesso potrebbe essere un risultato dovuto al simultaneo legame dell'anticorpo con la molecola bersaglio ma al tempo stesso con l'FcR presente sulla stessa cellula effettrice. Questo porta alla formazione di un complesso trimerico sulla cellula bersaglio, il cosiddetto "effetto scorpione" (8).

La esperienza acquisita finora vien arricchita da nuovi approcci immunoterapeutici, che prevedono la partecipazione guidata di cellule effettrici. Questo è l'obiettivo di uno sforzo collaborativo internazionale destinato al miglioramento degli attuali risultati. Non trascurabile è il fatto che queste forme terapeutiche raccolgono il gradimento dei pazienti, che si vedano sollevati dai pesanti effetti collaterali di chemioterapia (9).

## Bibliografia

1. Horenstein AL, Chillemi A, Quarona V, Zito A, Mariani V, Faini AC, Morandi F, Schiavoni I, Ausiello CM, Malavasi F.Antibody mimicry, receptors and clinical applications. Human Antibodies. 2017; 25: 75-85. Review.
2. Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, Vaisitti T, Aydin S. Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiological Reviews. 2008; 88: 841-886.
3. Malavasi F, Deaglio S, Damle R, Cutrona G, Ferrarini M, Chiorazzi N.CD38 and chronic lymphocytic leukemia: a decade later. Blood. 2011; 118: 3470-8.

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4. van de Donk NWCJ, Richardson PG, Malavasi F. CD38 antibodies in multiple myeloma: back to the future. Blood. 2018; 131: 13-29.
5. Malavasi F, Faini A. Anti-CD38 antibody in vivo therapy: not only target ligation [Commentary]. Clinical Cancer Research. 2019; (in press).
6. Morandi F, Horenstein AL, Costa F, Giuliani N, Pistoia V, Malavasi F. CD38: A Target for Immunotherapeutic Approaches in Multiple Myeloma. Front Immunol. 2018; 9: 2722.
7. Horenstein AL, Chillemi A, Zaccarello G, Bruzzone S, Quarona V, Zito A, Serra S, Malavasi F. A CD38/CD203a/CD73 ectoenzymatic pathway independent of CD39 drives a novel adenosinergic loop in human T lymphocytes. Oncoimmunology. 2013; 2: e26246.
8. Hogarth PM, Pietersz GA. Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. Nature Review Drug Discovery. 2012; 11: 311-331.
9. Ghobrial I, Cruz CH, Garfall A, Shah N, Munshi N, Kaufman J, Boise LB, Morgan G, Adalsteinsson V, Manier S, Pillai R, Malavasi F, Lonial S. Immunotherapy in multiple myeolma: accelerating on the path to the patient. Clinical Lymphoma, Myeloma and Leukemia. 2019; (in press).
10. Malavasi F, Faini AC. Mechanism of Action of a New Anti-CD38 Antibody: Enhancing. yeloma Immunotherapy. Clin Cancer Res. 2019 Mar 7. [Epub ahead of print]. ID: 30846477.
11. Ghobrial I, Cruz CH, Garfall A, Shah N, Munshi N, Kaufman J, Boise LH, Morgan G, Adalsteinsson VA, Manier S, Pillai R, Malavasi F, Lonial S.Immunotherapy in Multiple Myeloma: Accelerating on the Path to the Patient. Clin Lymphoma Myeloma Leuk. 2019 Feb 20. pii: S2152-2650(18)31257-6.
12. Manna A, Aulakh S, Jani P, Ahmed S, Akhtar S, Coignet M, Heckman M, Meghji Z, Bhatia K, Sharma A, Sher T, Alegria V, Malavasi F, Chini EN, Chanan-Khan A, Ailawadhi S, Paulus A. Targeting CD38 enhances the antileukemic activity of ibrutinib in chronic lymphocytic leukemia (CLL). Clin Cancer Res. 2019 Apr 2. pii: clincanres.3412.2018.

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# 15

# Adoptive cell therapy with anti-leukemia CTL for the prevention or treatment of leukemia relapse: from the bench to the bedside

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The prognosis for children affected by acute leukemia and transplanted in an advanced disease phase, in the presence of measurable minimal residual disease (MRD) or with unfavorable cytogenetic or molecular abnormalities is still poor. Based on retrospective data, the probability of relapse for high-risk patients often exceeds 50%. When relapse occurs after allogeneic hematopoietic stem cell transplantation (HSCT), in the majority of cases, only palliative therapy is possible (1, 2). Further intensification of pre-transplant chemotherapy and conditioning regimen would increase the incidence of treatment-related toxicity and non-relapse mortality. Thus, in the last years the clinical research has been directed towards the early identification of patients who cannot be cured by conventional treatment and who could benefit from the use of targeted therapy approaches (1, 3).

Cellular therapies approaches with donor-derived leukemia specific T cells may offer new treatment tools but their applicability has some restrictions. Limitations of these approaches include the identification of tumor-associated antigens with broad specificity, the ability of transferred cells to reach the tumor site, to display effector functions and to persist over time. A number of studies by our group and others demonstrated that naturally occurring patient-derived or donor-derived T and iNKT cells could emerge and have an important role in maintaining a state of remission in children affected by acute leukemia after chemotherapy alone or after allogeneic HSCT (4-8). Unfortunately, in the majority of cases they are probably insufficient in number or immunosuppressed by tumor immune evasion mechanism to efficiently exert their functions *in vivo*.

Adoptive immunotherapy with cytotoxic T lymphocytes (CTLs) directed against minor histocompatibility antigens or, more recently, against BCR-ABL peptides, have been successfully used to treat relapsed leukemia after SCT in adults (9-12), and represent a proof of principle of the potential efficacy of anti-leukemia T cell therapy with *ex vivo* expanded CTLs.

Results derived from clinical trials based on infusion of cytokine-induced killer (CIK) cells for the treatment of leukemia relapse in patients receiving allogeneic HSCT documented that these cells, induced by in vitro stimulation with IFNγ and IL-2, are able to mediate an anti-leukemia effect slightly superior to the



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unmanipulated donor-lymphocytes infusions (DLI), and low graft-versus-host-disease (GvHD) toxicity (13).

In recent years, we have optimized a procedure for generating and expanding CTLs directed against different types of tumor cells, including acute leukemia blasts (LB), through stimulation of peripheral blood mononuclear cell (PBMC) with dendritic cells (DC) pulsed with apoptotic patients' neoplastic cells in the presence of opportune cytokines. Donor-derived anti-leukemia CTLs displayed high levels of cytotoxicity against patients LB and negligible or low activity against patient-derived non-malignant cells, employed as an *in vitro* control to evaluate their potential alloreactivity capacity (14-16). Anti-leukemia CTL, generated using the whole tumor cells as source of leukemia-associated antigens (LAA), are likely to recognize a broader range of LAA, potentially reducing the risk of selecting variant leukemic subclones and include both effector and memory T-cells, suggesting the presence of lymphocytes able to exert, not only an immediate cytotoxic effector activity, but also to maintain long-term immune surveillance (17). Anti-leukemia CTL, produced in compliance with GMP requirements in the Cell factory of the IRCCS Policlinico San Matteo, were employed to prevent or treat leukemia relapse in pediatric patients receiving haploidentical HSCT (haplo-HSCT).

T-cell depleted, haplo-HSCT from partially matched family donor, offers an immediate transplant treatment, virtually to any patient in need of an allograft and lacking a suitable matched donor. Although the transfer of mature NK cells potentially able to mediate a graft versus leukemia effect, the relapse risk represents one of the major causes of failure of this treatment, in particular in the early post-transplant period due to the lack of mature T cells. One of the major advantages of using a family related donor is the possibility to collect additional cellular products from the same immediate available donor, which will not be rejected. For this reason, haplo-HSCT represents an ideal platform for post-transplant cellular therapy.

Then pediatric patients affected by acute myeloid (AML) or lymphoblastic leukemia (ALL) after haplo-HSCT, were treated so far, in the Pediatric Onco-hematology Unit of the IRCCS Policlinico San Matteo. Children received escalating or high CTL doses, based on disease state (presence of measurable levels of minimal residual disease or hematological relapse, respectively). Data obtained in these patients, whose follow up ranged between 9 months and 10 years, suggested that donor-derived anti-leukemia CTL might have a role in both prevention and treatment of post haplo-HSCT recurrence, also leading to long-term remission (18, 19). No severe adverse reactions, no grade 2-4 toxicities, including cytokine release syndrome and emergence of severe GvHD were recorded after CTL infusion. No differences were observed in response rate in ALL or AML patients, suggesting that anti-leukemia CTLs, directed against the whole leukemia blasts, may represent a valuable immunotherapeutic option for high-risk relapse patients. Protocol for prospective phase I-II study, for pediatric patients with high risk ALL or AML, given haplo-HSCT has been recently submitted to the National Regulatory Agency. The primary objective will be to evaluate the safety and

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tolerability of donor-derived CTL infusion for the prevention of leukemia relapse after haplo-HSCT, and the secondary objective was the evaluation of the efficacy of treatment in terms of prevention of relapse.

## Bibliografia


1. Lovisa F, Zecca M, Rossi B et al. Pre and post-transplant minimal residual disease predicts relapse occurrence in children with acute lymphoblastic leukaemia. Br J Haematol. 2018, in press.
2. Locatelli F, Masetti R, Rondelli R, et al. Outcome of children with high-risk acute myeloid leukemia given autologous or allogeneic hematopoietic cell transplantation in the AIEOP AML-2002/01 study. Bone Marrow Transplant. 2015; 50: 181-188.
3. Buldini B, Rizzati F, Masetti R, et al. Prognostic significance of flow-cytometry evaluation of MRD in children with acute myeloid leukaemia treated according to the AIEOP-AML 2002/01 study protocol. Br J Haematol. 2017; 177: 116-126.
4. Molldrem JJ, Lee PP, Wang C, et al. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nat Med. 2000; 6: 1018-1023.
5. Dhodapkar MV, Krasovsky J, Osman K, Geller MD. Vigorous premalignancy-specific effector T cell response in the bone marrow of patients with monoclonal gammopathy. J Exp Med. 2003; 198: 1753-1757.
6. Montagna D, Arico M, Montini E, De Benedetti F, Maccario R. Identification of HLA-unrestricted CD8+/CD28neg cytotoxic T-cell clones specific for leukemic blasts in children with acute leukemia. Cancer Res. 1995; 55: 3835-3839.
7. de Lalla C, Rinaldi A, Montagna D, et al. Invariant NKT cell reconstitution in pediatric leukemia patients given HLA-haploidentical stem cell transplantation defines distinct CD4+ and CD4- subset dynamics and correlates with remission state. J Immunol. 2011; 186: 4490-4499.
8. Dhodapkar MV, Richter J. Harnessing natural killer T (NKT) cells in human myeloma: progress and challenges. Clin. Immunol. 2011; 140: 160-166.
9. Yee C. Adoptive T cell therapy: points to consider. Current Opinion in Immunol. 2018: 51: 197-203.
10. Falkenburg JH, Wafelman AR, Joosten P, et al. Complete remission of accelerated phase chronic myeloid leukemia by treatment with leukemia-reactive cytotoxic T lymphocytes. Blood. 1999; 94: 1201-1208.
11. Warren EH, Fujii N, Akatsuka Y, et al. Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens. Blood. 2010; 115: 3869-3878.
12. Comoli P, Basso S, Riva G, et al. BCR-ABL-specific T-cell therapy in Ph+ ALL patients on tyrosine-kinase inhibitors. Blood. 2017; 129: 582-586.
13. Introna M and Correnti F. Innovative Clinical Perspectives for CIK Cells in Cancer Patients. Int J Mol Sci. 2018; 19.


TORNA ALL'INDICE

14. Montagna D, Maccario R, Locatelli F, et al. Ex vivo priming for long-term maintenance of antileukemia human cytotoxic T cells suggests a general procedure for adoptive immunotherapy. Blood. 2001; 98: 3359-3366.
15. Montagna D, Maccario R, Montini E, et al. Generation and *ex vivo* expansion of cytotoxic T lymphocytes directed toward different types of leukemia or myelodysplastic cells using both HLA-matched and partially matched donors. Exp Hematol. 2003; 11: 1031-1038.
16. Montagna D, Daudt L, Locatelli F, et al. Single-cell cloning of human, donor-derived antileukemia T-cell lines for in vitro separation of graft-versus-leukemia effect from graft-versus-host reaction. Cancer Res. 2006; 66: 7310-7316.
17. Daudt L, Maccario R, Locatelli F, et al. Interleukin-15 favors the expansion of central memory CD8+ T cells in ex vivo generated, antileukemia human cytotoxic T lymphocyte lines. J Immunother. 2008; 31: 385-393
18. Montagna D, Maccario R, Montini E, et al. Anti-leukemia CTL infusion for treatment of leukemia relapse in children given allogeneic hematopoietic stem cell transplantation. Bone Marrow Transpl. 2007; 39: S54-S55 (Suppl. 1).
19. Montagna D, Turin I, Ferulli F, et al. Safety and efficacy of anti-leukemia CTL infusions for prevention/treatment of leukemia relapse in high-risk patients given haploidentical HSCT. Haematologica. 2018; S3: S36-S37.

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# 16

# Improving CAR-T cell efficacy for solid tumours

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Le neoplasie solide rappresentano circa il 60% dei tumori maligni dell'età pediatrica. Importantissimi progressi sono stati raggiunti nel trattamento di molti di questi tumori negli ultimi due decenni, con il raggiungimento di una sopravvivenza media complessiva a 5 anni di circa il 78% (1, 2). Tuttavia, sebbene i bambini con malattia localizzata abbiano significativamente beneficiato di questo successo, quelli ad alto rischio, con malattia metastatica recidivante continuano ad avere una prognosi sfavorevole nonostante l'utilizzo delle nuove terapie convenzionali multimodali altamente aggressive. Queste ultime sono associate ad alti livelli di tossicità che aumento anche il tasso di insorgenza di malignità secondaria (3, 4). Inoltre, questi pazienti diventano spesso refrattari ad ulteriori terapie. Per questo motivo, c'è un grande bisogno di sviluppare nuove strategie anti-tumorali alternative meno tossiche e più efficaci. La crescente comprensione della biologia tumorale e l'interazione tra il tumore, il microambiente tumorale e il sistema immunitario, negli ultimi anni, ha portato a sviluppare nuove strategie immunoterapeutiche, capaci di attivare il sistema immunitario del paziente a combattere il cancro in un modo specifico, causando solo lievi tossicità. Diversi reports rilevano come molti tumori, tra cui anche quelli solidi, sembrano essere sensibili a queste forme d'immunoterapia, che comprendono l'uso di anticorpi monoclonali o bi-specifici, cellule del sistema immunitario come le cellule T o le natural killer e le terapie oncolitiche. È importante sottolineare, tuttavia, che la resistenza alle terapie convenzionali non sembra conferire alcuna resistenza alle terapie immunoterapeutiche(5). Per combinare l'effetto tumorale con quello cellula-mediato, le cellule T possono essere geneticamente ingegnerizzate per esprimere una nuova molecola chimerica di membrana nota come Recettore Chimerico Antigeno specifico (*"Chimeric Antigen Receptor"* - CAR), la quale combina la capacità specifica di legame di un anticorpo monoclonale con il dominio effettore intra-citoplasmatico del recettore delle cellule T (catena del CD3ζ) (6). Mentre importantissimi risultati sono stati ottenuti in pazienti affetti da malattie ematologiche maligne $CD19^{+}$ trattati con cellule T geneticamente modificate con un CAR specifico per tale l'antigene, come la leucemia linfoblastica acuta e i linfomi non-Hodgkin $CD19^{+}$[7], innumerevoli difficoltà per poter ottenere risposte simili si sono osservate nei pazienti affetti tumori solidi (8, 9). Infatti, sebbene molti studi clinici sono stati aperti negli ultimi anni anche nel setting dei tumori solidi con l'utilizzo di cellule CAR-T, i risultati non sono ancora



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soddisfacenti (10). Questo può essere facilmente spiegato dalla diversa natura di questi tumori con particolare riferimento al microambiente tumorale e ai meccanismi per bloccare e/o aggirare la risposta immunitaria. Infatti, è stato recentemente dimostrato che nel contesto dei tumori solidi la degradazione del Heparin Solfato, presente nella matrice extra-tumorale e nella membrana del basamento del microambiente tumorale, è un passaggio obbligato per ottenere un'efficacia clinicamente rilevante della terapia anti-tumorale con cellule CAR-T o antigene specifiche (11). Inoltre gli studi clinici condotti fino ad oggi evidenziano l'importanza della persistenza di queste cellule geneticamente modificate all'interno del paziente per mantenere e garantire la risposta anti-tumorale. Tuttavia, soprattutto nel setting dei tumori solidi, questo fenomeno è difficile da garantire (12). Evidenze mostrano come questo sia dovuto non solo alla qualità delle cellule infuse (13), ma anche al design del recettore chimerico e alla natura immunosopressiva del microambiente tumorale (14, 15). Strategie per implementare la risposta e la persistenza delle cellule T nel microambiente tumorale sono ora in fase di studio in diversi centri sia a livello pre-clinico che clinico (16, 17).

## Bibliografia


1. Mertens AC. Cause of mortality in 5-year survivors of childhood cancer. Pediatr Blood Cancer. 2007; 48: 723-726.
2. Smith MA, et al. Outcomes for children and adolescents with cancer: challenges for the twenty-first century. J Clin Oncol. 2010; 28: 2625-2634.
3. Neglia JP, et al. Second malignant neoplasms in five-year survivors of childhood cancer: childhood cancer survivor study. J Natl Cancer Inst. 2001; 93: 618-629.
4. Leung W, et al. Late effects of treatment in survivors of childhood acute myeloid leukemia. J Clin Oncol. 2000; 18: 3273-3279.
5. Cullen KV, Davey RA, Davey MW. Drug resistance does not correlate with resistance to Fas-mediated apoptosis. Leuk Res. 2001; 25: 69-75.
6. Caruana I, Diaconu I, Dotti G. From monoclonal antibodies to chimeric antigen receptors for the treatment of human malignancies. Semin Oncol. 2014; 41: 661-666.
7. Maude SL, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014; 371: 1507-1517.
8. Heczey A, Louis CU. Advances in chimeric antigen receptor immunotherapy for neuroblastoma. Discov Med. 2013; 16: 287-294.
9. Louis CU, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood. 2011; 118: 6050-6056.
10. Hartmann J, Schussler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med. 2017; 9: 1183-1197.
11. Caruana I, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. Nat Med. 2015; 21: 524-529.
12. Caruana I, et al. K562-Derived Whole-Cell Vaccine Enhances Antitumor Re-


TORNA ALL'INDICE

sponses of CAR-Redirected Virus-Specific Cytotoxic T Lymphocytes In Vivo. Clin Cancer Res. 2015; 21: 2952-2962.
13. Caruana I, Simula L, Locatelli F, Campello S. T lymphocytes against solid malignancies: winning ways to defeat tumours. Cell Stress. 2018; 2: 200-212.
14. Heczey A, et al. CAR T Cells Administered in Combination with Lymphodepletion and PD-1 Inhibition to Patients with Neuroblastoma. Mol Ther. 2017; 25: 2214-2224.
15. Quintarelli C, et al. Choice of costimulatory domains and of cytokines determines CAR T-cell activity in neuroblastoma. Oncoimmunology. 2018; 7: e1433518.
16. Nishio N, et al. Armed oncolytic virus enhances immune functions of chimeric antigen receptor-modified T cells in solid tumors. Cancer Res. 2014; 74: 5195-5205.
17. Bocca P, et al. Bevacizumab-mediated tumor vasculature remodelling improves tumor infiltration and antitumor efficacy of GD2-CAR T cells in a human neuroblastoma preclinical model. Oncoimmunology. 2017; 7: e1378843.

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# 17

# Graft-versus-leukemia and graft-versus-host disease: a possible balance?

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established therapeutic option for the treatment of a variety of hematologic malignancies, bone marrow failure states, and genetic diseases (1). After transplantation, donor-derived T cells provide many functions, such as enhancement of engraftment, protection from opportunistic infections, and, in the setting of malignancies, rejection of the underlying disease. However, the alloreactive T cells transferred with the stem cell graft, that respond to human leukocyte antigen (HLA) differences expressed on host tissues, may induce the graft-versus-host disease (GVHD), ranging from a mild skin rash to a life-threatening and in some instances life-ending complication (2-5). Although this posttransplantation complication is a significant cause of morbidity and mortality after allo-HSCT, GVHD has also a significant antitumor benefit, namely the graft-versus-leukemia (GVL) effect, a result of donor T cells capable of recognizing and rejecting residual malignant cells (6, 7). The GVL concept was developed from many lines of indirect evidence, including considerably lower relapse rates in patients who develop GVHD compared with rates in patients who do not develop this complication after transplantation, and the finding that T-cell depletion resulted in reduced risk of GVHD but also increased relapse rates (8). Therefore, the concept that the graft can exert antitumor effects is well established in the transplantation field and is a mainstay of the mechanism of how allo-HSCT can potentially cure patients with complex, often refractory, hematological malignancies. Despite this, a number of biological issues have not been fully elucidated, such as the cell subsets involved in GVL, the target antigens recognized on tumor cells, or the explanation for differences in GVL activity observed in the different hematologic malignancies. Thus, over the past several decades, attempts to identify and separate specific immune effector mechanisms that mediate GVHD and GVL have been conducted by several investigators.

One of the limitations in the application of allo-HSCT is the probability to find an HLA-matched family or unrelated donor, the ideal donors in terms of GVHD risk. In recent years, the use of alternative hematopoietic progenitor cell sources, including mismatched unrelated donors, umbilical cord blood, and haploidentical related donors, have been explored (9).

Transplantation from a full HLA-haplotype mismatched family member (haplo-HSCT), in addition to ensuring a donor for the large majority of patients, offers



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several other advantages, including prompt availability of the stem cell source, the possibility to select the best donor from a pool of family candidates, and immediate access to donor-derived cellular therapies either for the prevention of relapse or the treatment of infections after HSCT (10). Despite these advantages, widespread use of haplo-HSCT has been limited for many years by relevant complications mediated by bidirectional alloreactivity responsible for unacceptably high rates of graft rejection and severe GvHD. The continuous development of graft engineering and pharmacologic GVHD prevention strategies, together with better supportive care and optimal conditioning regimens, have significantly improved the outcomes of haploidentical HSCT, and this progress has led to establishment of haplo-HSCT as a standard therapeutic option for patients needing a HSCT procedure and lacking a HLA-identical or compatible donor (11, 12).

A major breakthrough intervened when preclinical studies in murine models demonstrated that infusion of large numbers of donor hematopoietic stem cells (HSC "mega dose") could overcome the major histocompatibility complex (MHC) barrier and promote engraftment (10). Seminal clinical studies by researchers in Perugia showed that transplantation of mega doses of stem cells, obtained by supplementing T cell-depleted bone marrow transplants with granulocyte colony-stimulating factor mobilized peripheral blood stem cells (PBSC), after a conditioning regimen consisting of total body irradiation (TBI), thiotepa, cyclophosphamide (CY) or fludarabine (FLU) and rabbit antithymocyte globulin (rATG), allowed for successful primary engraftment and relatively low incidence of acute and chronic GVHD despite the use of T-cell depletion as the only GVHD prophylaxis (12, 13). In the pediatric setting, different versions of the Perugia protocol were applied successfully to treat both malignant and non-malignant hematologic disorders (11,14). Despite acceptable rates of engraftment and GVHD, TRM due to infectious complications and malignancy relapse remained a major problem after CD34+ HSC-selected haplo-HSCT. In the attempt to ameliorate immune reconstitution, attempts were made to switch from positive CD34 HSC selection to negative T and B cell depletion, in order retain, besides the CD34+ stem cells, large numbers of other cells including γδ and NK cells, monocytes, and dendritic cells.

A more effective approach to negative depletion of T cells is the more recently described negative depletion of T-cell receptor (TcR) αβ+ T lymphocytes from mobilized peripheral stem cell grafts, coupled with B cell depletion. With this method, Bertaina et al. reported high OS and disease-free survival (DFS) (91%) coupled with a low incidence of acute GvHD (13%) and chronic GvHD in 23 children with a variety of non-malignant disorders (15). Recently, a multicenter Italian study comparing the outcome of T αβ/B cell depleted haplo-HSCT vs UD-HSCT in children with acute leukemia transplanted with a myeloablative regimen reported primary engraftment in 95 of 97 patients receving haplo-HSCT and, with the only pharmacologic GVHD prophylaxis of pretransplant ATG in the haplo-HSCT setting, 16% and 0% grade II-IV and III–IV acute GVHD, respectively, as compared to 39% and 12% in UD-HSCT recipients (16). After a median follow-up of 3.3 years, the 3-year leukemia-free survival was 63% vs 62% in the UD-HSCT setting, with chronic GVHD rates of 6% vs 20%, respectively.

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Different means to deplete alloreactive T cells within the graft have been experimented in the setting of haplo-HSCT. Triggering of alloreactivity in vitro through a mixed lymphocyte reaction (MLR) obtained by co-culturing donor T cells with recipient antigen-presenting cells has been generally followed by depletion of the activated donor T cells through surface activation markers or photoactive dyes (17,18). An alternative approach to prevent GVHD while preserving anti-leukemia and anti-infectious immunity is to functionally inactivate alloreactive T cells by inducing alloantigen-specific anergy (19, 20). Finally, coinfusion of CD4+CD25+ regulatory T cells (Tregs) and conventional donor T cells has been demonstrated to inhibit lethal GVHD after allogeneic HSCT across MHC, while preserving GVL surveillance both in animal models and humans (21, 22).

However, the best results in terms of enhancing GVL while keeping low GVHD rates have been obtained by T-cell depletion followed by infusion of controlled numbers of unmanipulated or antitumor-selected T cells. Proof of principle studies had demonstrated the feasibility to administer unmanipulated donor lymphocytes (DLI) to treat leukemia relapse after T-cell depleted HSCT (23). The rate of acute GVHD developing after the procedure, however, prompted manipulation of donor lymphocytes to reduce alloreactivity while maintaining immune surveillance potency. Two strategies have been explored to reduce the risks derived from alloreactivity associated with DLI. The first approach was based on transduction of nonspecific T cells with a retroviral construct containing suicide genes, to induce susceptibility to drug-mediated lysis in case of development of alloreactive response (24). Infusion of HSV-thymidine kinase gene-marked lymphocytes has proved safe and devoid of adverse effects (24). However, its mechanism of action requires interference with DNA synthesis so that cell killing may take several days and be incomplete, resulting in a delay in clinical benefit. Recently, an alternative strategy that relies on inducible caspase proteins (iCasp9) to exploit the mitochondrial apoptotic pathway has been explored. The use of DLI modified by iCasp9 cell-suicide system in a small cohort of children transplanted for acute leukemia demonstrated the potential advantages in terms of rapid and consistent cell removal in case of GVHD development (25). Escalating doses of iCasp9-modified DLI have been employed in 20 pediatric patients receiving T αβ depleted haplo-HSCT for PID, and proved safe (25% cumulative incidence of aGVHD, no TRM) and able to provide prompt immune reconstitution (26).

An alternate strategy consists in delivering infectious/leukemia antigen-specific T cells selected by cell culture or by sorting. Attempts have been made to boost tumor-specific responses and control leukemia relapse by post-transplant add-backs of donor cytotoxic T cells (CTLs) directed towards patients blasts (27,28), minor histocompatibility antigens (29), or leukemia-related antigens (30). One of the main limitations is that CTL antigen recognition is major histocompatibility complex (MHC)- restricted. Moreover, in many cases, tumor-specific antigens able to elicit protective immune responses have not been identified.

To extend the recognition specificity of T lymphocytes beyond their classical MHC-peptide complexes, a gene-therapeutic strategy has been developed that allows redirecting T cells to defined tumor cell surface antigens, by the transfer of an

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antigen-binding moiety, most commonly a single chain variable fragment derived from a monoclonal antibody, together with an activating T-cell receptor (chimeric antigen receptors, CARs) (31). Recently, CARs directed to the CD19 molecule, expressed on B-cell malignancies, have been employed in pediatric and adult patients with refractory ALL and proven highly efficient, with CR rates of 70% to 90% (reviewed in 32). These studies included patients with a prior history of allogeneic HSCT, and no GVHD was recorded. It has been shown that leukemia blasts may escape immune control mediated by T cells and cause relapse by losing HLA mismatched alleles after HSCT, due to an acquired uniparental disomy, with consecutive total loss of the HLA-mismatched haplotype (33). In this case, infusion of selected and/or activated NK cells may help control leukemia relapse.

**Bibliografia**

1. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006; 354: 1813-1826.
2. Ferrara JLM, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009; 373: 1550-1561.
3. Petersdorf EW. Which factors influence thedevelopment of GVHD in HLA-matched or mismatched transplants? Best Pract Res Clin Haematol. 2017; 30: 333-335.
4. Shlomchik WD. Graft-versus-host disease. Nat Rev Immunol. 2007; 7: 340-352.
5. Zecca M, Prete A, Rondelli R, Lanino E, Balduzzi A, Messina C, Fagioli F, Porta F, Favre C, Pession A, Locatelli F. Chronic graft-versus-host disease in children: incidence, risk factors, and impact on outcome. Blood. 2002; 100: 1192-1200.
6. Riddell SR, Berger C, Murata M, Randolph S, Warren EH. The graft versus leukemia response after allogeneic hematopoietic stem cell transplantation. Blood Rev. 2003; 17: 153-162.
7. Dickinson AM, Norden J, Li S, Hromadnikova I, Schmid C, Schmetzer H, Kolb H-J. Graft-versus-Leukemia Effect Following Hematopoietic Stem Cell Transplantation for Leukemia. Front Immunol. 2017 Jun 7; 8: 496.
8. Negrin RS. Graft-versus-host disease versus graft-versus-leukemia. Hematology Am Soc Hematol Educ Program. 2015; 2015: 225-230.
9. Rocha V, Locatelli F. Searching for alternative hematopoietic stem cell donors for pediatric patients. Bone Marrow Transplant. 2008; 41: 207-214.
10. Martelli MF, Aversa F. Haploidentical transplants using ex vivo T-cell depletion. Semin Hematol. 2016; 53: 252-256.
11. Zecca M, Comoli P. Haploidentic SCT Applications in pediatric patients. In: T. Demirer, ed. Progress in Stem Cell Transplantation. In-Tech pub. ISBN 978-953-51-2227-2¸ Doi: 10.5772/59336
12. Aversa F, Tabilio A, Velardi A, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. N Engl J Med. 1998; 339: 1186-1193.

TORNA ALL'INDICE

13. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. J Clin Oncol. 2005; 23: 3447-3454.
14. Pende D, Marcenaro S, Falco M, et al. Antileukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. Blood. 2009; 113: 3119-3129.
15. Bertaina A, Merli P, Rutella S, et al. HLA-haploidentical stem cell transplantation after removal of αβ+ T and B cells in children with nonmalignant disorders. Blood. 2014; 124: 822-826.
16. Bertaina A, Zecca M, Buldini B, et al. Unrelated donor vs HLA-haploidentical α/β T-cell- and B-cell-depleted HSCT in children with acute leukemia. Blood. 2018; 132: 2594-2607.
17. Montagna D, Yvon E, Calcaterra V, et al. Depletion of alloreactive T cells by a specific anti-interleukin-2 receptor p55 chain immunotoxin does not impair in vitro antileukemia and antiviral activity. Blood. 1999; 93: 3550-3557.
18. Amrolia PJ, Muccioli-Casadei G, Huls H, et al. Adoptive immunotherapy with allodepleted donor T-cells improves immune reconstitution after haploidentical stem cell transplantation. Blood. 2006; 108: 1797-1808.
19. Comoli P, Montagna D, Moretta A, Zecca M, Locatelli F, Maccario R. Alloantigen-induced human lymphocytes rendered nonresponsive by a combination of anti-CD80 monoclonal antibodies and cyclosporin-A suppress mixed lymphocyte reaction in vitro. J Immunol. 1995; 155: 5506-5511.
20. Guinan EC, Boussiotis VA, Neuberg D, Brennan LL, Hirano N, Nadler LM, Gribben JG. Transplantation of anergic histoincompatible bone marrow allografts. N Engl J Med. 1999; 340: 170414.
21. Edinger M, Hoffmann P, Ermann J, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat Med. 2003; 9: 1144-1150.
22. Di Ianni M, Falzetti F, Carotti A, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. Blood. 2011; 117: 3921-3928.
23. Klingebiel T, Handgretinger R, Lang P, Bader P, Niethammer D. Haploidentical transplantation for acute lymphoblastic leukemia in childhood. Blood Rev. 2004; 18: 181-192.
24. Ciceri F, Bonini C, Stanghellini MT, et al. Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I-II study. Lancet Oncol. 2009; 10: 489-500.
25. Di Stasi A, Tey SK, Dotti G, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N Engl J Med. 2011; 365: 1673-1683.
26. Kapoor N, Bertaina A, Merli P, et al. Outcome of Children with Primary Immune-Deficiencies (PIDs) enrolled in a phase I-II trial based on the infusion of BPX-501 donor t cells genetically modified with a novel suicide gene (induc-

TORNA ALL'INDICE

ible Caspase 9, iC9) after T-cell depleted HLA-haploidentical allogeneic stem cell transplantation (haplo-HSCT). Blood. 2016; 128: 4683.
27. Falkenburg JH, Wafelman AR, Joosten P, et al. Complete remission of accelerated phase chronic myeloid leukemia by treatment with leukemia-reactive cytotoxic T lymphocytes. Blood. 1999; 94: 1201-1208.
28. Daudt L, Maccario R, Locatelli F, et al. Interleukin-15 favors the expansion of central memory CD8+ T cells in ex vivo generated, antileukemia human cytotoxic T lymphocyte lines. J Immunother. 2008; 31: 385-393.
29. Warren EH, Fujii N, Akatsuka Y, et al. Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens. Blood. 2010; 115: 3869-3878.
30. Comoli P, Basso S, Riva G, et al. BCR-ABL-specific T-cell therapy in Ph+ ALL patients on tyrosine-kinase inhibitors. Blood. 2017; 129: 582-586.
31. Smith M, Zakrewski J, James S, Sadelain M. Posttransplant chimeric antigen receptor therapy. Blood. 2018; 131: 1045-1052.
32. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018; 359: 1361-1365.
33. Vago L, Perna SK, Zanussi M, et al. Loss of mismatched HLA in leukemia after stem-cell transplantation. N Engl J Med. 2009; 361: 478-488.

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