

Immune Cells as Targets and Tools for Cancer Therapy

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#Contributed Equally

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Commentary

Immune cells are deeply intertwined in the development and progression of cancer. Their physiological functions can be divided into two main aspects on one hand they respond against the tumour and on the other they can counteract these responses or even promote tumour growth. For that reason immune cells are an obvious tool of therapeutic interventions in cancer treatment. Successful use of checkpoint inhibitors and cell-based therapies in the last few years has rapidly increased interest in manipulation of immune cell for cancer therapy. In this commentary we review the potential of targeting or using of macrophages, T cells, dendritic cells and NK cells in the cancer therapy.

Macrophages as Drug Targets

Macrophages representing the major component of the infiltrate of solid tumours are called tumour-associated macrophages (TAMs). In contrast to inflammatory macrophages, TAMs are described as “alternatively” activated macrophages as they undergo stimulation by cytokines and chemokines that are present in the tumour microenvironment including IL-4, IL-10 and CCL2. TAMs show low tumoricidal activity and they promote tissue remodelling and angiogenesis. TAMs also promote tumour development, its local invasion and spread to distant sites [1] (Sica and Mantovani, 2012), thus may constitute as an interesting target for anti-cancer therapy. Particularly blockade of the tumour infiltration by TAM can inhibit tumor growth or progression. For example, blocking of the colony stimulating factor 1 receptor (CSF-1R) in mice with aggressive mammary cancer, reduces pulmonary metastases regulated by macrophages (Figure 1) [2] (DeNardo et al., 2011). Furthermore, CCL2-blocking antibody reduces number of metastasis and prolongs the survival of tumour-bearing mice [3] (Qian et al., 2011). Whereas, manipulation of Wnt signalling can change TAM activation state and thereby impact tumour progression [4] (Król et al., 2014). Application of class IIa Histone Acetyl Deacetylase (HDAC) inhibitors, increased tumour infiltration by CD11b⁺ cells, eventually differentiating into macrophages. The treatment had positive effect on tumour-vasculature, whereas reduced cancer cell proliferation [5] (Guerriero et al., 2017). Deletion of REDD1 gene in hypoxic TAMs increases glucose uptake and changes the macrophage metabolism towards glycolysis. This, causes competition between TAMs and tumour endothelial cells, and results in stabilization of tumour vasculature and reduction of metastasis [6] (Geeraerts et al., 2017; [7] Wenes et al., 2016). An alternative approach to this is stimulating macrophages to increase T cell immune response [8] (Tseng et al., 2013). This was done using anti-CD47 antibody, which increased phagocytosis of cancer cells by macrophages and increased priming of CD8⁺ T cells, accompanied by

decreased priming of CD4⁺ T cells. Overall, reprogramming methods including: antibody-mediated inactivation of IL-10, delivery of Toll-like receptor (TLR) agonists and targeting intracellular signalling molecules in combination with T cell-enhancing checkpoint inhibitors, allows changing the pro-tumoural immune infiltrate to an anti-tumoural state. These results indicate that modulated macrophages may represent as an interesting option for novel immune therapy.

Engineered T cells in Cancer Treatment

T cells play a key role in cell-mediated immunity that is why for over fifty years they have been investigated to harness their potential in cancer therapy. Tumour-infiltrating lymphocytes can generate antitumour immune responses and are normally associated with a positive prognosis; however immunosuppression can hamper its effects. Adoptively transferred T cells have the potential to target and destroy cancer cells, while engineered T cells can overcome tumour immune evasion [9] (Pegram et al., 2012). Nevertheless, transferred autologous T cells are inefficient in completely rejecting a tumour [10] (Mackensen et al., 2006; [11] Yee et al., 2002). Expression of novel, genetically engineered receptors in T cells, improves cells persistence after adoptive transfer and enhance tumour specificity. Two main types of T cell modification exist. The first is the expression of cloned tumour antigen-specific T cell receptor (TCR) that is expressed next to the endogenous TCR. For that, α and β chains of TCR are identified, isolated and transduced into recipient T cells. In combination with identification of patient-specific mutations, this technology can be used to tailor anticancer responses according to patients' tumour genetic makeup. The second type is the expression of chimeric antigen receptors (CARs), with high specificity for antigen recognition in an MHC-independent fashion (Figure 1) [12] (Fesnak et al., 2016). Furthermore, antigens recognized by CAR T cells do not need to be peptides, but can also be a glycolipids or carbohydrates. Since the first generation of CAR T cells, an enormous improvement has been made. Second and third generation of CARs contain not only the main ζ domain of TCR, but also one or more costimulatory domains of CD28, ICOS, or 41BB which provide complete activation signals for T cells. Such CAR T cells are not only more effective in antigen recognition but also in stimulation of proliferation, survival and resistance to T cell anergy [13] (Gill and June, 2015).

B cell malignancies are the most commonly targeted tumour types by engineered T cells. Firstly, they are relatively common and express several conserved cell surface markers (e.g. CD19,CD20,CD22). Furthermore, circulating B cell tumours provide an easy access for engineered T cells that are intravenously infused. CD19 is the most common B cell target for engineered T cell therapies. is expressed almost exclusively on benign and most malignant B cells [14]

(Scheuermann and Racila, 1995) and therefore, possible “on-target off-tumour” activity of cytotoxic T cells are limited [15] (Maude et al., 2014).

However a common complication of CAR T cell therapy is the cytokine release syndrome (CRS), but this can be controlled with an efficient treatment e.g. anti-interleukin-6 receptor antibody [15] (Maude et al., 2014). The main factors that influence efficacy of genetically modified T cell therapy are the loss of the targeted antigen expression or the inability to sustain their persistence and activation [16] (Yang et al., 2005). An additional challenge remaining is the efficiency of tumour targeting by CAR T cells in solid tumours, where poor tissue penetration is a major problem. This is illustrated by the observation that local administration of CAR T cells had higher accumulation at tumour sites compared with the systemic administration [17] (Parente-Pereira et al., 2011).

To be efficient most immune anticancer responses must overcome the immunosuppressive tumour microenvironment. Strategies that remove or deplete suppressor cells in combination with adoptive T cell therapy might enhance anti-tumour responses in cancer immunotherapies. Checkpoint inhibitors act as immunological brake-removers and tumour cells can utilise these checkpoints to escape destruction by the immune system. The addition of programmed cell death protein 1 (PD1) monoclonal antibody enhanced the anti-tumour effects of CAR T cells in preclinical models, suggesting that engineered T cells like other immune cells undergo immune suppression[18] (John et al., 2013). Future therapies will most likely be accompanied by

multiple immunomodulatory modalities, as checkpoint inhibition, to further enhance their efficacy. An alternative immunostimulatory approach is the combination of engineered T cells with inhibitors of immunosuppressive enzymes such as arginase or indoleamine 2,3-dioxygenase (IDO) or other checkpoint regulators such as VISTA or CD200R [19] (Nowak et al., 2017 [20] Rygiel and Meyaard, 2012) . However safety of such combination treatments would need to be carefully verified, particularly in view of current safety challenges of the CAR T cells technology. Additional concern of the use of engineered T cells is the off-target reactivity and cytokine-release syndrome (CRS). Rapid tumour clearance has been associated with CRS that occasionally leads even to fatalities. Engineering of gene-edited human CAR T cells have been shown using techniques, including CRISPR-Cas9, molecular ‘switches’ enabling greater control over the performance of engineered T cells *in vivo* and may improve safety [21] (Ren et al., 2017). Cells may be engineered to express pro-death signals that can be induced with an exogenous element. An example of the “off switch” is the inducible human caspase 9, leading to deletion of CAR T cells in the animal model [22] (Gargett and Brown, 2014). Other strategies that improve safety are: generation of T cells with a dual CAR that recognizes two targets or cells with expression of target molecules for monoclonal antibodies that could be used to eliminate CAR T cells when necessary [12] (Fesnak et al., 2016). Successful clinical trials with genetically modified T cells provide a proof of principle that shows the potential of the “living drug” therapies. However, the challenges remaining are broader effectiveness in solid tumours and better regulation to increase safety.

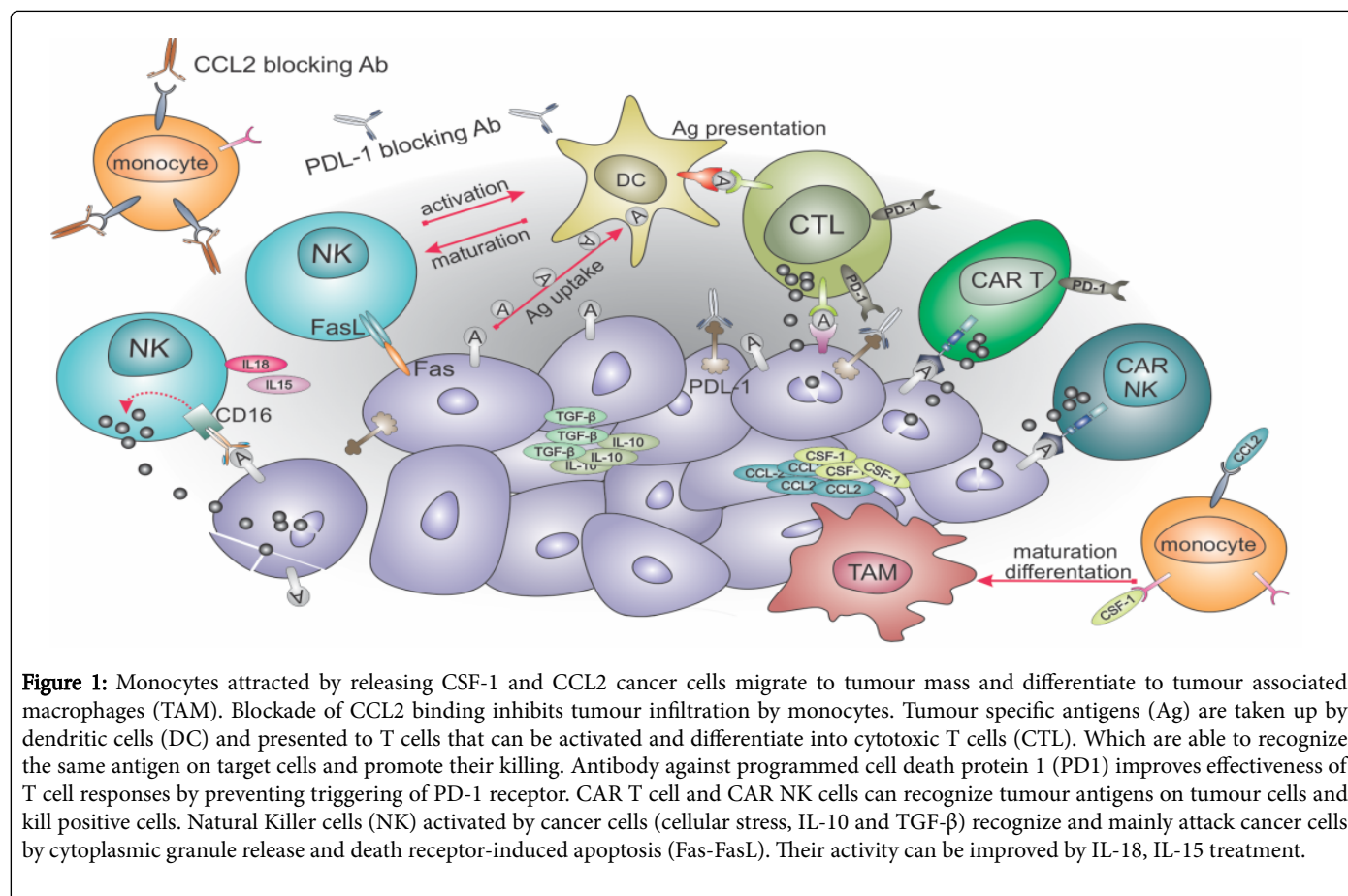


Figure 1: Monocytes attracted by releasing CSF-1 and CCL2 cancer cells migrate to tumour mass and differentiate to tumour associated macrophages (TAM). Blockade of CCL2 binding inhibits tumour infiltration by monocytes. Tumour specific antigens (Ag) are taken up by dendritic cells (DC) and presented to T cells that can be activated and differentiate into cytotoxic T cells (CTL). Which are able to recognize the same antigen on target cells and promote their killing. Antibody against programmed cell death protein 1 (PD1) improves effectiveness of T cell responses by preventing triggering of PD-1 receptor. CAR T cell and CAR NK cells can recognize tumour antigens on tumour cells and kill positive cells. Natural Killer cells (NK) activated by cancer cells (cellular stress, IL-10 and TGF-β) recognize and mainly attack cancer cells by cytoplasmic granule release and death receptor-induced apoptosis (Fas-FasL). Their activity can be improved by IL-18, IL-15 treatment.

Dendritic Cells in Therapy

Dendritic cells (DCs) are at the center of the immune system owing to their ability to control both immune tolerance and to react against cancer. DCs are an essential component of vaccination because of their capacity to capture, process, and present antigens by MHC class I molecules to enable antitumour CD8⁺ T cell activation (Figure 1). DC-based immunotherapy can be used for vaccination against cancer through various ways in targeted peptide/protein and nucleic-acid-based vaccines captured by DCs *in vivo* [23] (Boon et al., 2006), vaccines composed of DCs and antibodies conjugated with antigens [24] (Palucka and Banchereau, 2012; [25] Tel et al., 2013) and vaccines composed of *ex-vivo*-generated DCs that are loaded with antigens. Sipuleucel-T (Provenge) is the first DC-based immunotherapy approved for the treatment of hormone-refractory prostate cancer [26] (Plosker, 2011). The next step from *ex vivo*-engineered DCs is to target DCs *in vivo* in patients (*in vivo* DCs targeting) [27] (Macri et al., 2016). Several methods have been explored to target DCs *in vivo*. These include tumour-associated antigens (TAA)-bound antibodies targeting receptors on DCs [28] (Dhodapkar et al., 2014), oncolytic viruses expressing GM-CSF, CD40L and other immune stimulatory molecules such as bacterial proteins (HP-NAP) [29] (Ramachandran et al., 2014). An antibody against a molecule expressed on DC (DEC205) tagged with NY-ESO-1, co-administered with TLR (an agonist as adjuvants) is currently in a phase-I trial and it has been shown to be feasible and safe [28] (Dhodapkar et al., 2014). Another attractive strategy is to generate and use oncolytic viruses secreting GM-CSF. The foundation for this strategy is that viral oncolysis will release neoantigens from the cancer cells that can be captured by the tumour-residing DCs, which are then activated by virus-derived GM-CSF. An oncolytic Herpes Simplex virus secreting GM-CSF (T-Vec) was recently approved for the treatment of melanoma. Another tool to improve immunostimulatory potential of DCs is to target immunosuppressive molecule such as PD-L1, CTLA-4 or IL-10 to neutralize inhibitory signals. Several preclinical tumour models show that CTLA-4 blockade after DC vaccination may indeed enhance DC vaccine-induced T-cell responses [30] (Pierret et al., 2009; [31] Ribas et al., 2009 [32] Tarhini and Iqbal, 2010). In addition, it has been shown that DC-based immunotherapy in combination with anti-CTLA-4 antibodies seems to be more effective than the use of these agents alone [31] (Ribas et al., 2009; [33] Wilgenhof et al., 2016). Currently, there is no evident clinical data on the combination of DC vaccination with anti-PD-1 antibodies, but they are under investigation and the first results are expected in the near future. Similarly as in the case of engineered T cells, the safety of such combinatorial treatments will require a thorough investigation to reduce side effects.

Vaccine-induced protective immunity can be modulated using strategies that reduce or block other inhibitory molecules such as, CD200 to enhance the T cell activation. It has been demonstrated that lack of CD200R signaling inhibits outgrowth of endogenous tumours [34] (Rygiel et al., 2012). An interesting way to enhance the CD200-CD200R blockade is to combine the blocking agent with TLR7 stimulation. This goal is encouraged by studies showing that, a lack of CD200 increases TLR7-dependent immune response [35] (Karnam et al., 2012). Several other TLR ligands are currently being tested in clinical trials, including CpG oligodeoxynucleotides (ODNs) (TLR9 ligand) and polyinosinic-polycytidylic acid (polyI:C) (TLR3 ligand) [36] (Shi et al., 2016).

NK cells in tumour immune-surveillance and therapy

Natural killer (NK) cells are lymphocytes of the innate immune system, known for their ability to recognize and kill malignant cells even in the absence of preimmunization or stimulation. Thus, making NK cells another promising target for cell-based immunotherapy for cancer treatment. Despite the fact that NK cells represent only 5-15% of circulating human lymphocytes, they exert immediate antitumour effect by releasing cytolytic enzymes, inducing FasL and TRAIL-mediated apoptosis and antibody-dependent cellular cytotoxicity (ADCC) (Figure 1). Many tumours have developed strategies to escape the immune surveillance by NK cells. This may be due to inhibition of NK cells by self- histocompatibility antigens (i.e. HLA-G) or suppressive signals (i.e. NKG2D ligands) derived from tumours and tumour-associated immune cells, such as myeloid-derived suppressor cells (MDSC), Tregs and TAMs [37] (Costello et al., 2002; [38] Urosevic and Dummer, 2008; [39] Waldmann, 2006; [40] Weil et al., 2017). The impaired expression of natural cytotoxicity receptors (NCRs) and defective function of NK cells was observed in many haematological malignancies and solid tumours [37] (Costello et al., 2002; [41] Dahlberg et al., 2015). To increase the cytotoxic potential of NK cell-based therapies, new approaches have evolved towards the use of expanded or *ex vivo* stimulated allogeneic NK cells, NK cell lines and genetically engineered NK cells with increased expression of cytokines, antibody binding receptors and activating receptors. The most extensively studied cytokine utilized to expand and activate NK cells is IL-2. Antitumour potential of IL-2 stimulated NK cells was documented in clinical trials in patients with advanced metastatic cancers [42] (Rosenberg et al., 1985). However, use of IL-2 as an activating agent may also trigger Tregs proliferation via stimulation of the receptors for IL-2 and IL-2R α (CD25). Selective depletion of CD25⁺ cells by lymphodepleting agents and IL-2 fused with diphtheria toxin (IL-2DT) followed by haploidentical NK cells infusion increased complete remission rate in AML patients in comparison to standard IL-2 administration [43] (Bachanova et al., 2014). IL-15 is another cytokine essential for NK cell maturation and survival, which has demonstrated a marked synergy with IL-2 on the viability and proliferation of NK cells [44] (Sieglar et al., 2010). Unlike IL-2, IL-15 does not induce Treg cell proliferation and capillary leak syndrome, thus IL-15 may be safely administered with NK cells [39] (Waldmann, 2006; [45] Waldmann et al., 2011). Preactivation of NK cells with a cocktail of cytokines such as IL-12, IL-15, and IL-18 demonstrated an increase in antitumour effects [46] (Leong et al., 2014). Initial clinical trials suggest that NK-cell based therapy is safe and feasible; nevertheless, there is still a need to optimize the manufacturing process of clinical grade NK-cell products. Early investigations using cell lines derived from NK cells, such as the NK-92 cell line has revealed cytotoxic activity towards a wide range of malignant cells and has been used as a source of NK cells in clinical trials [47] (Arai et al., 2008; [48] Klingemann and Miyagawa, 1996; [49] Yan et al., 1998). The advantage of NK-92 cell line is the lack of inhibitory receptors such as KIRs and NKp44 [50] (Maki et al., 2001). On the other hand, NK-92 cells do not express Fc γ RIIIa receptor (CD16), thus are unable to mediate ADCC, an important mechanism of action for the use of anti-cancer antibodies in therapy. This defect can be reverted by the introduction of mRNA coding for the antibody-binding receptor CD16. CD16-expressing variants could be combined with antitumour monoclonal antibodies in the clinic [51] (Carlsten et al., 2016; [52] Clémenceau et al., 2013).

Finally, the design of CAR-modified NK cells have enabled high affinity specific recognition of tumour antigens and enhanced the

cytotoxic potential of NK cells and NK cell lines in antitumour therapies, especially targeting B-cell malignancies (anti-CD19, anti-CD20 CARs) [53] (Boissel et al., 2013; [54] Glienke et al., 2015). CAR-based immunotherapy have been used also in solid tumours, such as neuroblastoma (anti-GD2 CAR) and breast cancer (anti-EpCAM CAR) [55] (Altvater et al., 2009; [56] Sahn et al., 2012). Her-2, which is overexpressed in many types of human malignancies also represents as a promising target for CAR-expressing NK cell-based therapies. Further studies clarifying the antitumour potential of modified NK cells have to be initiated, in order to maximize their cytotoxic potential in NK cell-based therapies.

Conclusions

The combination of standard or novel anti-cancer therapy together with targeting of immune cells present in the tumour microenvironment constitutes the future of cancer treatment. Our current understanding of tumour development and interactions occurring between cancer cells and immune cells enables for more efficient immune manipulations. A parallel change of immunosuppressive tumour microenvironment with stimulation of effector cell-based responses will be the best combination of immunotherapy.

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