Teaming up against cancer: tumour immunology, immunotherapy and CARTs

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Cancer is among the four main causes of death. Despite all our efforts, therapies for advanced cancer still show poor effectiveness: late-stage cancer patients are still faced with a *complete remission* rate of only about 5-10%.

Decades of experience have proven the central role of the *immune system* in the fight against cancer, which has yielded some promising *immunotherapies* that allow us to *team up* with our own immune system. The journal Science named tumour immunotherapy *science Breakthrough of the Year 2013* and chimeric antigen receptor-modified T cells (*CARTs*), an innovative strategy that combines the *virtues* of previous immunotherapies with the limitless potential of *synthetic biology*, are one of the main reasons for Science's decision.

Objectives and methods

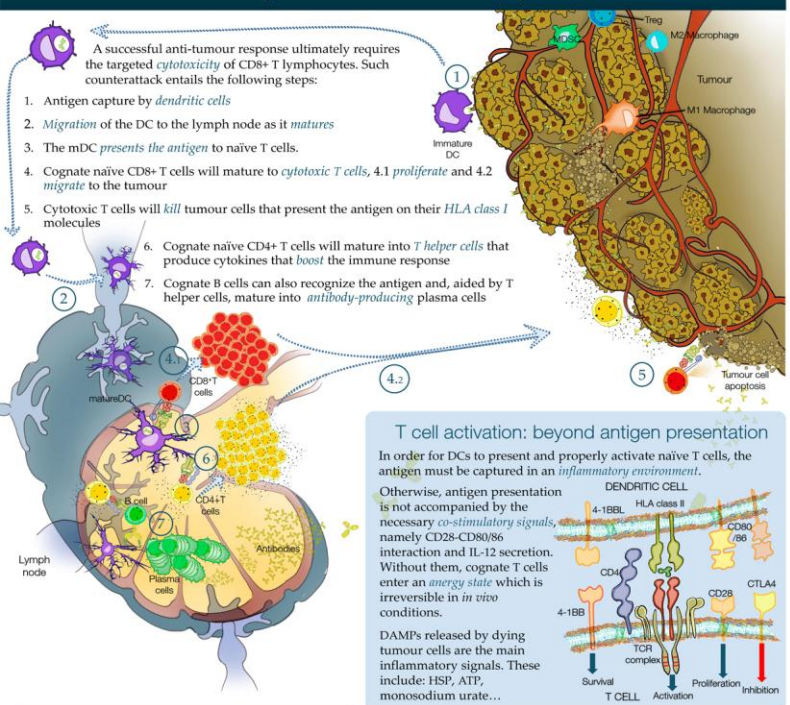
Current literature has been consulted and reviewed in order to accomplish the following goals:

First, we briefly describe the *immune response* against tumours, providing a framework to mention the most relevant tumour immunotherapies that helped set the *foundations* for the development *CARTs*. We describe CARTs with detail, enumerating their *virtues* and their *downsides*. We cover their *engineering* and *components*, and how synthetic biology can help us *improve* their performance and reduce their undesired effects. We have an overlook at their *current* clinical *status*. Finally, we try to grasp what the *future* holds for this promising tumour immunotherapy.

The biological war against tumours: immunity and immunotherapy

A successful anti-tumour response ultimately requires the targeted *cytotoxicity* of CD8+ T lymphocytes. Such counterattack entails the following steps:

1. Antigen capture by *dendritic cells*
2. *Migration* of the DC to the lymph node as it *matures*
3. The mDC *presents the antigen* to naïve T cells.
4. Cognate naïve CD8+ T cells will mature to *cytotoxic T cells*, 4.1 *proliferate* and 4.2 *migrate* to the tumour
5. Cytotoxic T cells will *kill* tumour cells that present the antigen on their *HLA class I* molecules
6. Cognate naïve CD4+ T cells will mature into *T helper cells* that produce cytokines that *boost* the immune response
7. Cognate B cells can also recognize the antigen and, aided by T helper cells, mature into *antibody-producing* plasma cells



T cell activation: beyond antigen presentation

In order for DCs to present and properly activate naïve T cells, the antigen must be captured in an *inflammatory environment*.

Otherwise, antigen presentation is not accompanied by the necessary *co-stimulatory signals*, namely CD28-CD80/86 interaction and IL-12 secretion. Without them, cognate T cells enter an *anergy state* which is irreversible in *in vivo* conditions.

DAMPs released by dying tumour cells are the main inflammatory signals. These include: HSP, ATP, monosodium urate…

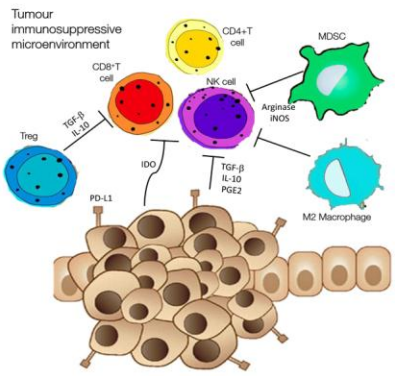
Tumour evasion of the immune response

Tumour cells are capable of developing mechanisms to evade the immune response but they are not alone. Tumour *stromal cells* also provide a favourable environment for the tumour.

Evasion mechanisms include:

Hiding from effector T cells
- Down-regulation of *antigen* expression
- Down-regulation of *HLA* expression
- Accumulation of errors in the antigen processing pathway

Hindering the immune system
- Immunosuppressive *paracrine secretion*: TGF-β, PGE2, IL-10 and VEGF
- Surface expression of membrane *ligands* that induce T cell *anergy* and *exhaustion* such as PD-L1 and CTLA-4
- Secretion of CCL2 and CXCL12 which *lure in* MDSC, inducers of heavy immunosuppression
- Hypoxia leads to the release of CCL28 which *attracts* Treg cells
- Macrophage alternative maturation to the *immunosuppressive phenotype* M2 is favoured



Aiding our immune system: Immunotherapy

Coley's Toxin: the origin of immunotherapy

In the late XIX century, William B. Coley, a bone surgeon, came across the man on the picture, whose recurrent cheek sarcoma showed a miraculous complete remission after an infection had ensued at the site of the cancer. He believed that the *immune response* unleashed by the infection was behind the recovery and he went on to craft a vaccine using immunogenic bacterial extracts: Coley's toxin, which set the foundations for tumour immunotherapy.

What is Immunotherapy?

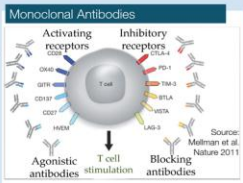
Immunotherapy refers to any type of treatment that relies on manipulation of our immune system by either *boosting*, *suppressing* or *modulating* it.

Though many promising results have been accomplished, we are still far from complete success.

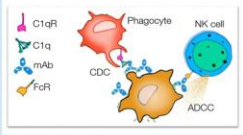
The two immunotherapies on the right, monoclonal antibodies and adoptive T cell transfer, are the basis of CART therapy

Source: Balkwill. Nature Reviews 2009

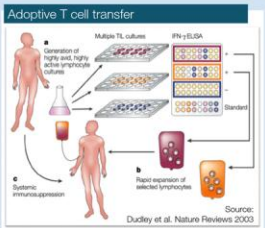
Monoclonal Antibodies



Monoclonal antibodies can be used to stimulate T cell activity as shown on the upper image. But they can also target tumour antigens to unleash CDC or ADCC.



Adoptive T cell transfer



Adoptive T cell transfer can be summarised as the inoculation of tumour affected patients with immunologically reactive T cells capable of mediating either directly or indirectly an anti-tumour activity.
Tumour infiltrating lymphocytes are obtained from surgically removed tumours, selected in vitro, activated, expanded and re-infused back into the patient, with a high dose of IL-2.

One step further in immunotherapy: Chimeric Antigen Receptor-modified T cells

CARTs combine our best strategies in immunotherapy with synthetic biology

More than two decades ago, 1st generation chimeric antigen receptors (CARs) were conceived. They were fusion membrane proteins combining: an extracellular domain made of the single-chain variable fragment (scFv) from an antibody and an intracellular domain containing the TCR complex component in charge of transducing the activating signal, CD3ζ.

These receptors were transfected into T cells and adoptively transferred back into the patient.

The scFv region conferred the CAR transfected T cell (CART) the specificity to interact with a specific antigen, in this case a tumour antigen. Then, upon CAR—antigen interaction, the CD3ζ would activate the T cell and induce its targeted cytotoxicity.

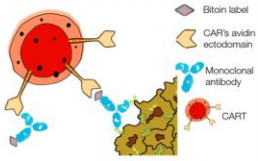
First clinical results were not as promising as expected, however, 2nd and 3rd generation CARs were developed ushering in a new era in immunotherapy

Engineering the ultimate immunological weapon

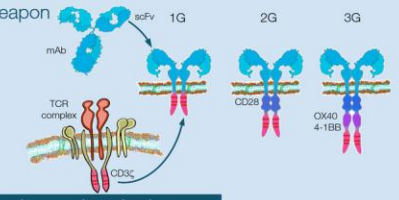
CARs' structure can be divided in 3 domains and all of them play a part in the CAR's performance:

Ectodomain

It is the region of antigen recognition and interaction. It is most commonly made from the scFv region of an antibody with specificity for a certain antigen. *First*, a tumour antigen to be targeted has to be identified. *Secondly* , using a *hybridoma* bank, a proper cognate mAb has to be identified. *Finally* the mAb has to be sequenced.



CARs can also be engineered with an *avidin* ectodomain which is specific for *biotin*. Hence, any mAb labelled with biotin can be used directly *in vivo*, avoiding having to craft a new CAR molecule for each patient.



Hinge and transmembrane domain

The structure of these regions impacts the *efficiency* of the CAR molecule, although the exact mechanism is not know. Until we do, researchers have to *empirically* test different alternatives in each case. Options for the hinge include: CH3 or CH2CH3.

Endodomain

It is the region in charge of activating intracellular pathways.

It always incorporates an activating CD3ζ motif, and 3rd generation CARTs also include two or more motifs that induce proliferation (CD28) and survival (4-1BB, OX40).

New options include motifs that induce *cytokine* release. These CARs act eliminating the *immunosuppressive* microenvironment generated by the tumour, favouring DC *maturation* after antigen capture and *rewiring* tolerizing immune cells such as MDSCs and M2 macrophages.

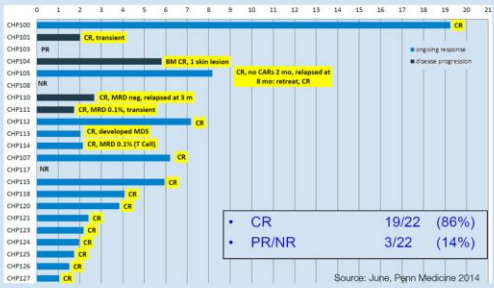
CARTs achieve their first victory

Most promising results CARTs have offered are in the treatment of haematological malignancies, especially in acute lymphoid leukaemia.

The opposite chart shows the results obtained as of November of 2013 by a clinical trial run at The Children's Hospital of Philadelphia.

19 of the 22 patients treated showed clinical responses and 15 achieved complete remission which was on-going at the time.

Many more clinical trials are being carried out. Solid tumours, however, are proving more resistant to CART therapy, but researchers are hopeful of the possibilities in this field.



CARTs: Friends and foes

Virtues

- HLA-independent, highly effective, immunotargeted cytotoxicity
- Higher avidity than TCR. Avidity can also be controlled
- Long *in vivo* persistence
- Use of synthetic biology allow us to re-wire the internal pathways and genetic elements of the effector cell to adjust many aspects of its performance.

Downsides, difficulties and concerns

- Antigen identification is the hardest part. It must be:
  - A membrane protein
  - Absent on healthy vital tissues
  - Essential to the tumour cell so that it cannot down-regulate its expression
- Its protocol is very difficult to standardize making inter-centre clinical trials extremely challenging.
- Tumour immunosuppressive microenvironment can hamper the activity of CARTs. However, strategies have been suggested to tackle this problem.
- Off-tumour toxicity can be expected and relies on the antigen targeted
- On-target toxicity, including cytokine release syndrome and tumour lysis syndrome cam ensue. While treatment for the latter exists, the former is still hard to tackle, trials using an anti-cytokine mAb, Tocilizumab, are underway.

FINAL REMARKS: THE SKY IS THE LIMIT

CARTs are proof that we have come a long way since Colley crafted the first vaccine to help boost the immune system in its war against tumours, and current achievements of synthetic biology make it unfathomable how much further we will go.

Immunotherapy has produced some fascinating strategies in the treatment of tumours, although with modest results. CARTs come as an effort to combine the virtues of several previous treatments to make up for their flaws and restrictions. In this regard, CARTs combine the unsurpassable specificity and MHC-independent activity of mAbs with the activation, expansion and survival signals of TCRs, CD28 and 4-1BB respectively, thus creating adoptively transferred T cells capable of targeted cytotoxicity that can also be engineered to bypass tumour's immunosuppressive effects.

Although this seems hypothetically flawless, only limited by the identification of the appropriate antigen to be targeted, early clinical assays are beginning to show researchers the aspects that need to be improved; however, promising results, especially in the treatment of haematological malignancies, let us think that we should be able to eventually succeed.

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Abbreviations

ADCC Antibody-Dependent Cell-mediated Cytotoxicity
C1q Complement component C1q
C1qR Complement component C1q Receptor
CCL2/28 Chemokine CC Ligand-2/28
CD Cluster of Differentiation
CDC Complement-Dependent Cytotoxicity
CH2/3 Heavy chain constant domain 2/3
CTLA-4 Cytotoxic T-Lymphocyte Antigen 4
CXCL12 Chemokine CXC Ligand-12
DC Dendritic Cell
FcR Fragment crystallisable Receptor
HLA Human Leukocyte antigen
IL Interleukin
mAb Monoclonal Antibody
MDSC Myeloid Derived Suppressor Cell
PD-L1 Programmed Death Ligand 1
PGE2 Prostaglandin-E2
TCR T-cell receptor
TGFb Transforming Growth Factor
VEGF Vascular endothelial growth factor