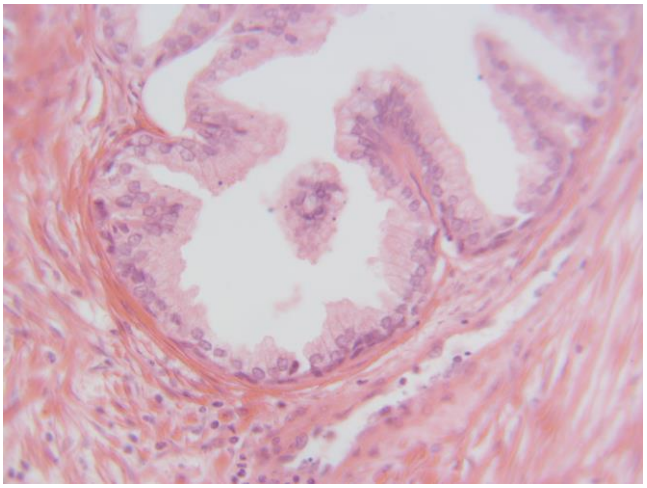

REPROGRAMMING AND CANCER

TGL 201

ABSTRACT

Reprogramming is the process of taking one cell type and changing it to another. In this Note we examine some recent reprogramming of cancer cells into macrophages and thus having cancer specific antigen presenting cells, unique to the specific originating malignancy.

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NOTICE

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Furthermore, this document contains references to and quotes and modified charts and figures from papers and documents under the premise of “Fair Use” in order to present ideas and understandings in context. The Author has attempted to make any and all references to such material separate from those of the author per se and has referenced the source expressly in all cases. These documents are for the dissemination of ideas and have no commercial intent. Our approach herein is to take elements of what is recent in the literature focused on a specific topic and attempt to develop a tapestry image of these connectable elements. We do not necessarily provide any new or fundamental results but merely attempt to assemble elements in a systematic and holistic manner.

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Contents

1 INTRODUCTION

The immune system has been employed in a variety of ways to attack cancers. Key to this approach is an ability to recognize a cancer cell and thus activate the immune response. Over the past decades we have seen a variety of means to address the identification problem of cancer cells. In fact a malignant cell mass may very well have a multiplicity of different markers on different cells. Thus a cell by cell marker process may become necessary.

In this Note we examine the concept of reprogramming, namely the ability to take one somatic mature cell and convert it to another cell. In this case we examine two extremes. One is the Nobel Prize winning taking of a fibroblast and convert it to a pluripotent cell. The second is a recent work converting a malignant cell to an immune cell with the ability to express the tumor antigens.

Reprogramming can go in several directions. As we noted from a somatic to an embryonic and from a somatic to a somatic. There are many challenges here. Fundamentally all cells allegedly contain a full complement of DNA. But as cells mature many genes are silenced in one way of another and thus somatic cells become somewhat unique in that they express and how they act. Reprogramming is an attempt to alter that commitment by reinserting genes and activating them to change the cell from one type to another. The full logical and scientific basis for these reinsertions however appears limited.

1.1 RECENT RESULTS

Let us first begin with the cancer cell transition. As Linde et al have noted:

Therapeutic cancer vaccination seeks to elicit activation of tumor-reactive T cells capable of recognizing ***tumor-associated antigens (TAA)*** *and eradicating malignant cells.*

Here, we present a cancer vaccination approach ***utilizing myeloid-lineage reprogramming*** *to* ***directly convert cancer cells*** *into* ***tumor-reprogrammed antigen-presenting cells (TR-APC).***

Using syngeneic murine leukemia models, we demonstrate that TR-APCs:

1. *acquire both myeloid phenotype and function,*

2. *process and present endogenous TAAs, and*

3. *potently stimulate* ***TAA-specific CD4+ and CD8+ T cells.***

In vivo TR-APC induction elicits clonal expansion of cancer-specific T cells, establishes cancer-specific immune memory, and ultimately promotes leukemia eradication.

We further show that both hematologic cancers and solid tumors, including sarcomas and carcinomas, are amenable to myeloid-lineage reprogramming into TR-APCs.

Finally, we demonstrate the clinical applicability of this approach by generating TR-APCs from primary clinical specimens and stimulating autologous patient-derived T cells. Thus, TR-APCs represent a cancer vaccination therapeutic strategy with broad implications for clinical immuno-oncology.

Despite recent advances, the clinical benefit provided by cancer vaccination remains limited. We present a cancer vaccination approach leveraging myeloid-lineage reprogramming of cancer cells into APCs, which subsequently activate anticancer immunity through presentation of self-derived cancer antigens.

Both hematologic and solid malignancies derive significant therapeutic benefit from reprogramming-based immunotherapy.

From Stanford Medicine we have the following[1]:

Some cities fight gangs with ex-members who educate kids and starve gangs of new recruits. Stanford Medicine researchers have done something similar with cancer ***— altering cancer cells so that they teach the body's immune system to fight the very cancer the cells came from.***

"This approach could open up an entirely new therapeutic approach to treating cancer," said Ravi Majeti, MD, PhD, a professor of hematology and the study's senior author. The research was published March 1 in Cancer Discovery.

The lead author is Miles Linde, PhD, a former PhD student in immunology who is now at the Fred Hutchinson Cancer Institute in Seattle.

Some of the most promising cancer treatments use the patient's own immune system to attack the cancer, often by taking the brakes off immune responses to cancer or by teaching the immune system to recognize and attack the cancer more vigorously. T cells, part of the immune system that learns to identify and attack new pathogens such as viruses, can be trained to recognize specific cancer antigens, which are proteins that generate an immune response.

For instance, in CAR T-cell therapy, T cells are taken from a patient, programmed to recognize a specific cancer antigen, then returned to the patient. But there are many cancer antigens, and physicians sometimes need to guess which ones will be most potent.
Like an immune response

A better approach would be to train T cells to recognize cancer via processes that more closely mimic the way things naturally occur in the body *— like the way a vaccine teaches the immune system to recognize pathogens. T cells learn to recognize pathogens because special antigen*

[1] https://med.stanford.edu/news/all-news/2023/03/cancer-hematology.html

presenting cells (APCs) gather pieces of the pathogen and show them to the T cells in a way that tells the T cells, "Here is what the pathogen looks like — go get it."

Something similar in cancer would be for APCs to gather up the many antigens that characterize a cancer cell. That way, instead of T cells being programmed to attack one or a few antigens, they are trained to recognize many cancer antigens and are more likely to wage a multipronged attack on the cancer.

Now that researchers have become adept at ***transforming one kind of cell into another****, Majeti and his colleagues* ***had a hunch that if they turned cancer cells into a type of APC called macrophages****, they would be naturally adept at teaching T cells what to attack.*

"We hypothesized that maybe ***cancer cells reprogrammed into macrophage cells could stimulate T cells because those APCs carry all the antigens of the cancer cells they came from****," said Majeti, who is also the RZ Cao Professor, assistant director of the Institute for Stem Cell Biology and Regenerative Medicine and director of the Ludwig Center for Cancer Stem Cell Research and Medicine.*
Cell conversion

The study builds on prior research from the Majeti lab ***showing that cells taken from patients with a type of acute leukemia could be converted into non-leukemic macrophages with many of the properties of APCs****.*

In the current study, the researchers programmed mouse leukemia cells so that some of them could be induced to transform themselves into APCs. When they tested their cancer vaccine strategy on the mouse immune system, the mice successfully cleared the cancer.

"When we first saw the data showing clearance of the leukemia in the mice with working immune systems, we were blown away," Majeti said. "We couldn't believe it worked as well as it did."

Other experiments showed that the cells created from cancer cells were indeed acting as antigen-presenting cells that sensitized T cells to the cancer. "What's more, we showed that the immune system remembered what these cells taught them," Majeti said. "When we reintroduced cancer to these mice over 100 days after the initial tumor inoculation, they still had a strong immunological response that protected them."

"We wondered, If this works with leukemias, will it also work with solid tumors?" Majeti said. The team tested the same approach using mouse fibrosarcoma, breast cancer, and bone cancer. "The transformation of cancer cells from solid tumors was not as efficient, but we still observed positive results," Majeti said. With all three cancers, the creation of tumor-derived APCs led to significantly improved survival.

Lastly, the researchers returned to the original type of acute leukemia. When the human leukemia cell-derived APCs were exposed to human T cells from the same patient, they observed

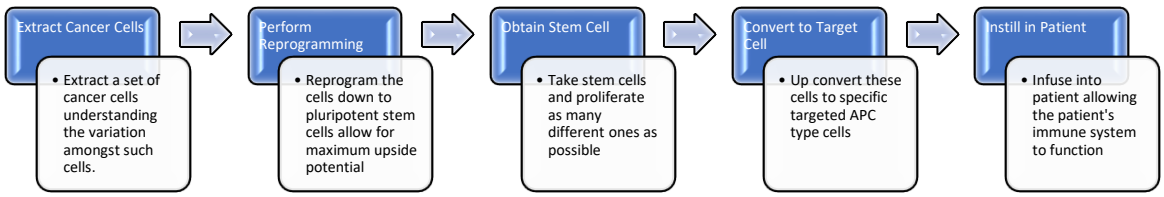
all the signs that would be expected if the APCs were indeed teaching the T cells how to attack the leukemia.

"We showed that reprogrammed tumor cells could lead to a durable and systemic attack on the cancer in mice and a similar response with human patient immune cells," Majeti said. "In the future we might be able to take out tumor cells, transform them into APCs and give them back to patients as a therapeutic cancer vaccine."

"Ultimately, we might be able to inject RNA into patients and transform enough cells to activate the immune system against cancer without having to take cells out first," Majeti said. "That's science fiction at this point, but that's the direction we are interested in going."

1.2 THE PARADIGM

We can consider several paradigms for the use of reprogramming. The example below considers an amalgam of both approaches.



This approach is an amalgam, namely taking both reprogramming efforts and attempting to ensure maximal efficacy.

1.3 VACCINE?

The use of a reprogramming approach has by some been considered a vaccine approach. Historically a vaccine is generally considered as some entity which primes the immune system to subsequently attack cells or other entities that reflect that target entity, and the targeting is some specific known entity. In contrast, reprogrammed cells are entities that prime the immune system by their very nature, using targets which may not be known and using targets that may have a multiplicity of antigen like targets. Perhaps the difference may be subtle yet the problem of many vaccines is that they are second order primers. Reprogrammed cells are first order primers. We shall try to establish this detail as we go through the analysis.

1.4 OVERVIEW

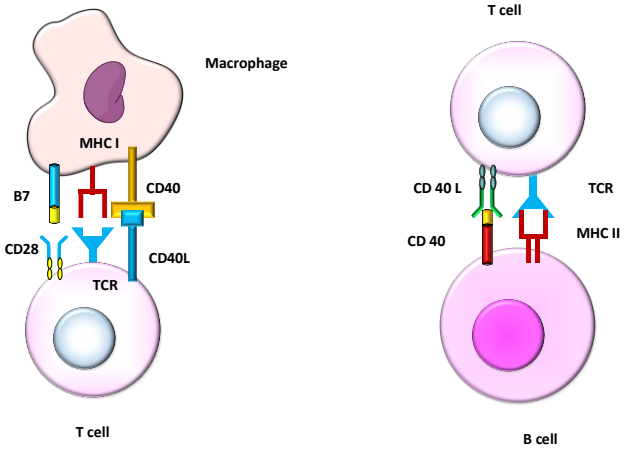
Let us summarize what we will discuss herein.

1. Antigen Presenting Cells: APC are cells which present antigens, Ag, to immune system cells. Having said that it begs the question of just what is an antigen and exactly how is this recognition made. Ag for the most park are fragments of some intruding cell which has been collected by the APC, dendrite or macrophage for example, and then placed on an MHC protein on the surface of the APC and made available to an immune cell. Frankly there is still a great deal of arm waving here since we must be able to determine what an Ag is and how it interacts both with the MHC and the receptors on the immune cells. By that we mean if the Ag is a protein sequence then what is the affinity issues between all these other proteins.
2. Macrophages: The macrophage is the end cell associated with the example we focus on herein. Macrophages are complex and have a variety of forms. It is likely as we learn more about them the subtle differences will become clear and essential to the functions. We examine these issues as best as one can at this stage. Like all the other issues it is a moving target.
3. Phagocytosis: We examine the attack on the cells from which the Ags are obtained.
4. Macrophages and Cancer: Macrophages can play various roles in cancer. M1 are cancer attacking and M2 varieties protect the cancer cells. Thus a choice of a macrophage as in the cases discussed may present a challenge sing the cells may morph from one kind to another. This process has not yet been examined.
5. Reprogramming: We discuss reprogramming in the various types. Reprogramming first produced stem cells. Now they allow cell morphing. We discuss these in some detail.
6. Pluripotential Reprogramming: This is the Nobel Prize approach reducing a somatic cell to a stem cell.
7. Lateral Reprogramming: This approach takes one somatic cell into another type.
8. Vaccines: Here we discuss cancer vaccines. It begs the question as to whether the laterally reprogrammed cells are a form of vaccine or an adjunct to such.
9. Observations: As with other Notes we present several observations. However in this case many of the observation relate to the unknowns regarding the process. Reprogramming begs answers to the process for which we have little to none.

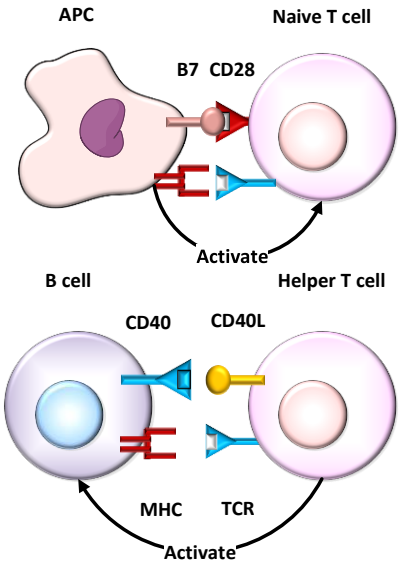
2 APC

Antigen presenting cells, APC, are cells which are part of the immune system and have the capability of priming such cells as T cells to identify and attack specific targeted cells. Macrophages are considered as APCs. However as we attempt to dig deeper into understanding the reprogramming of tumor cells it is worth examining both the APC construct and more importantly the macrophages.

We begin with a brief summary of the key elements of the cells in question. First below we show the fundamental T cell and macrophage construct and the subsequent T and B cell interfaces.



The specific interactions that we see are shown below:



We now will consider the above elements in further detail as relates to cancers.

2.1 APC CONSTRUCTS

We start with an understanding of the antigen presenting cells. As Eiz-Vespera and Schmetzer note:

The immune system is permanently confronted with mutated and self-, microbe-, and tumor-derived neoantigens – as well as other, "unknown" antigens – and has to differentiate between self or nonself.

These antigenic molecules (protein or lipid based) must be phagocytosed, processed, and/or presented in the respective major histocompatibility complex (MHC) molecules on the cell surface in recognizable form to train immune cells such as effector T cells, leading to their specific activation. These "trainers" are so-called antigen-presenting cells (APCs), which can be divided into professional (e.g., dendritic cells [DCs], B cells, and macrophages) and nonprofessional APCs (e.g., fibroblasts and hepatocytes).

While all nucleated human cells can present peptide fragments of endogenous proteins using the MHC class I pathway and display them on the surface to CD8+ cytotoxic T lymphocytes, only professional APCs such as DCs, macrophages, and B cells are characterized by the ability to present exogenous antigens using MHC class II molecules and present them on MHC class II molecules to CD4+ T-helper cells (TH cells), along with the required costimulatory molecules, such as CD86 and CD83 molecules.

Therefore, the main difference between professional and nonprofessional APCs is the absence of MHC class II and costimulatory molecules on nonprofessional APCs. Recently, it was described that the three main granulocyte subsets (neutrophils, eosinophils, and basophils) also seem to be able to present exogenous antigens to naive TH cells via MHC class II molecules, which has led to the suggestion that they should be referred to as APCs.

Only professional APCs provide all three signals (antigen presentation via MHC molecules, expression of costimulatory molecules, and cytokine/chemokine secretion) needed to train and activate T cells to recognize, destroy, or tolerate cells that carry these antigens, and thereby to control viral infections or cancer cell growth.

Macrophages and DCs internalize pathogens and cellular debris by phagocytosis, whereas B cells use the Bcell receptor for antigen uptake.

Antigens are presented to T cells along with the required costimulatory molecules to get activated, get "licensed" to mediate their (helper or cytotoxic) function, and produce memory cells. DCs are most effective at presenting tumor and viral antigens of intracellular origin because they have the ability to "cross-present" antigens.

A variety of DC subtypes in various organs with different phenotypical and functional characteristics mediating wound healing, proinflammation, or anti-infectious or antitumor attack were described, and they can be used for immune profiling to monitor the grade of activation or suppression of the immune system. Plasmacytoid or special tolerogenic DCs regulate responses of the innate and adaptive immune cells and contribute to avoiding autoimmune reactions. DC-based treatments have been applied for almost three decades and so

far have been tested most often in patients with malignant melanoma, prostate cancer, malignant glioma, or renal cell cancer. DCs were also applied in combination with cytokine-induced killer cells to treat gastrointestinal tumors, lung cancer, and breast cancer…

Abbas et al have defined the key terms as follows:

Antigen: A molecule that binds to an antibody or a TCR. Antigens that bind to antibodies include all classes of molecules. Most TCRs bind only peptide fragments of proteins complexed with MHC molecules; both the peptide ligand and the native protein from which it is derived are called T cell antigens[2].

and also:

Antigen-presenting cell (APC): A cell that displays peptide fragments of protein antigens, in association with MHC molecules, on its surface and activates antigen-specific T cells. In addition to displaying peptide-MHC complexes, APCs also express costimulatory molecules to optimally activate T lymphocytes.

We now begin to reflect on reprogramming of somatic cells from type to type using the work of Majeti. As Majeti describes in the Patent:

An antigen-presenting cell (APC) or accessory cell is a cell that displays antigen complexed with major histocompatibility complexes (MHCs) on the cell surfaces; this process is known as antigen presentation.

T cells can recognize these complexes using their T cell receptors. While almost all cell types can present antigens in some way, the co-stimulatory molecules and MHC antigens present on professional antigen-presenting cells, including macrophages, B cells and dendritic cells, present foreign antigens to both helper T cells, including naive T cells, as well as cytotoxic T cells; and provide for an enhanced response.

Characteristics of professional APCs include phagocytosis or receptor-mediated endocytosis of antigen, processing the antigen into peptide fragments and then displaying those peptides, bound to a class II MHC molecule, on their membrane*.*

The T cell recognizes and interacts with the antigen-class II MHC molecule complex on the membrane of the antigen presenting cell. An additional co-stimulatory signal can be produced by

[2] From Abbas et al: *Proteins that are present in the cytosol are degraded by proteasomes to yield peptides that are displayed on class I MHC molecules, while proteins that are ingested from the extracellular environment and sequestered in vesicles are degraded in lysosomes (or late endosomes) to generate peptides that are presented on class II MHC molecules. Thus, the site of proteolysis is the key determinant of which MHC molecules, class I or class II, the generated peptides will bind to. As we have discussed previously, the function of CD8+ CTLs is to kill cells producing foreign antigens in the cytosol, and the function of CD4+ T cells is to activate macrophages and B cells, which may have ingested microbes and protein antigens. The pathways of antigen processing play a key role in determining the types of microbes and protein antigens that these classes of T cells recognize and respond to. We first describe these two pathways of antigen processing and then their functional significance.*

the antigen-presenting cell, leading to activation of the T cell. The expression of co-stimulatory molecules and MHC class II are defining features of professional APCs

Macrophage: A tissue-based phagocytic cell derived from fetal hematopoietic organs or blood monocytes that plays important roles in innate and adaptive immune responses. Macrophages are activated by microbial products such as endotoxin and by T cell cytokines such as IFN-γ. Activated macrophages phagocytose and kill microorganisms, secrete proinflammatory cytokines, and present antigens to helper T cells. Macrophages in different tissues are given different names and may serve special functions; these tissue macrophages include the microglia of the central nervous system, Kupffer cells in the liver, alveolar macrophages in the lung, and osteoclasts in bone.

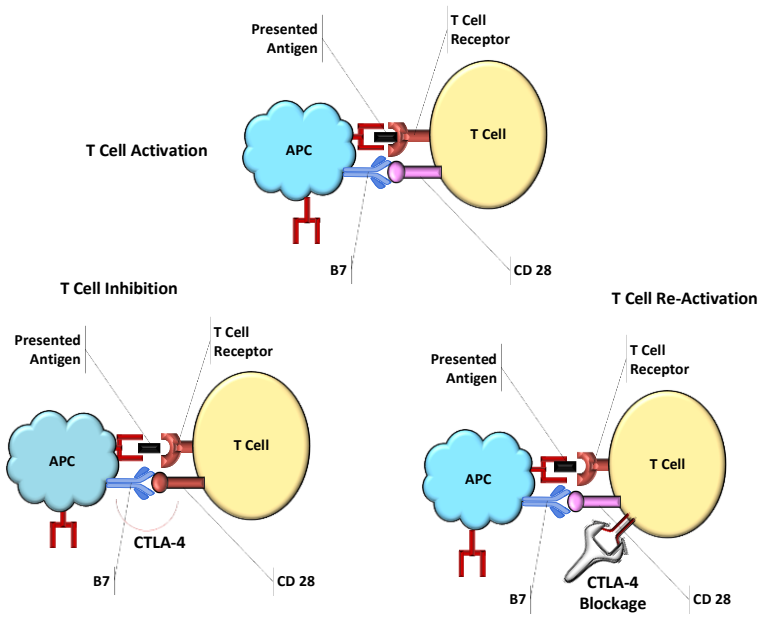
Major histocompatibility complex (MHC) molecule: A heterodimeric membrane protein encoded in the MHC locus that serves as a peptide display molecule for recognition by T lymphocytes. Two structurally distinct types of MHC molecules exist. Class I MHC molecules are present on most nucleated cells, bind peptides derived from cytosolic proteins, and are recognized by CD8+ T cells. Class II MHC molecules are restricted largely to dendritic cells, macrophages, and B lymphocytes, bind peptides derived from endocytosed proteins, and are recognized by CD4+ T cells.

Cytotoxic (or cytolytic) T lymphocyte (CTL): A type of T lymphocyte whose major effector function is to recognize and kill host cells infected with viruses or other intracellular microbes. CTLs usually express CD8 and recognize microbial peptides displayed by class I MHC molecules. CTL killing of infected cells involves delivery of the contents of cytoplasmic granules into the cytosol of infected cells, leading to apoptotic death.

Different cell types function as antigen-presenting cells to activate naive T cells or previously differentiated effector T cells. DCs are the most effective APCs for activating naive T cells and therefore for initiating T cell responses. DCs … functions in innate immunity … Macrophages and B lymphocytes also function as APCs, but mostly for previously activated CD4+ helper T cells rather than for naive T cells.

DCs, macrophages, and B lymphocytes express class II MHC molecules and other molecules involved in stimulating T cells and are therefore capable of activating CD4+ T lymphocytes. For this reason, these three cell types have been called professional APCs; however, this term is sometimes used to refer only to DCs because of their unique role in naive T cell activation. The following Table is a summary of such cells.

Cell Type	Class II Major Histocompatibility Complex	Costimulators	Principal Function
Dendritic cells	Constitutive; increases with maturation; increased by IFN-γ and T cells (CD40L-CD40 interactions)	Constitutive; expression is increased with TLR signals, IFN-γ, CD40-CD40L interactions	Antigen presentation to naive T cells in initiation of T cell responses to protein antigens (priming)
Macrophages	Low or negative; increased by IFN-γ and T cells (CD40L-CD40 interactions)	Expression is increased by TLR signals, IFN-γ, CD40-CD40L interactions	Antigen presentation to effector CD4+ T cells in effector phase of cell-mediated immune responses (T cell–enhanced killing of phagocytosed microbes)
B lymphocytes	Constitutive; increased by IL-4, antigen receptor cross-linking, and T cells (CD40L-CD40 interactions)	Expression is increased by T cells (CD40-CD40L interactions), antigen receptor cross-linking	Antigen presentation to CD4+ helper T cells in humoral immune responses (helper T cell–B cell interactions)
Vascular endothelial cells	Inducible by IFN-γ; constitutive in humans	Low; may be inducible	May promote activation of antigen-specific T cells at site of antigen exposure and in organ grafts
Various epithelial and mesenchymal cells	Inducible by IFN-γ	Probably none	No known physiologic function; possible role in inflammatory diseases



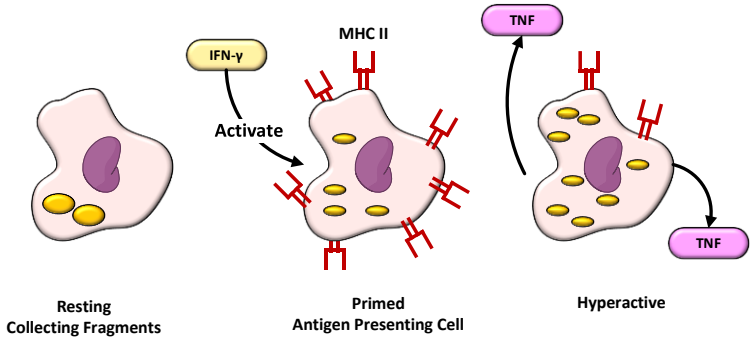
2.2 MACROPHAGES

Macrophages are really a set of complex cells which classed as a macrophage exhibit often dramatically disparate characteristics. We will attempt to re-examine the macrophage in the context of reprogramming.

Macrophages as part of the M1 set generally have three states. They are:

1. Resting: where the cell just collects cellular remains but remains inactive
2. Primed: The cell had been primed by the immune system by IFN-γ and MHC II are prepared.
3. Hyperactive: here the cell become a true killer sending out TNF to eliminate invaders.

We demonstrate this below:



From Abbas et al we have similar definitions:

Macrophages are capable of both inhibiting and promoting the growth and spread of cancers, depending on their activation state. Classically activated M1 macrophages can kill many types of tumor cells. How macrophages are activated by tumors is not known. A possible mechanism is recognition of damage-associated molecular patterns from dying tumor cells by macrophage innate immune receptors.

Macrophages in tumors also may be activated to kill tumor cells by IFN-γ produced by tumor-specific Th1 cells, CTLs, and NK cells. This may be why a large number of Th1 cells in some tumors is correlated with a good prognosis. M1 macrophages can kill tumor cells by mechanisms that they also use to kill infectious organisms, including the liberation of lysosomal enzymes, nitric oxide, and reactive oxygen species, ….M2 macrophages promote tumor growth ….

We shall also examine M2 types in some detail later. Now from Zhang et al:

Macrophages are very plastic cells with different phenotypes and functions, which are impacted both by their origin and resident tissue microenvironment.

Broadly, macrophages can be activated into two distinct subsets based on the M1/M2 paradigm, classically activated or M1 macrophages and alternatively activated or M2 macrophages .

M1 macrophages are polarized in vitro by Th1 cytokines such as colony-stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), and interferon-γ (IFN-γ) alone or together with lipopolysaccharide (LPS) from bacteria. M1 macrophages express pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6, IL-12, IL-23, and TNF-α .

In contrast, M2 macrophages are polarized by Th2 cytokines such as IL-4 and IL-13 and produce anti-inflammatory cytokines such as IL-10 and transforming growth factor beta (TGF-β) .

Recent studies have revealed that the M1/M2 paradigm is not sufficient to encompass all states of macrophage activation. Macrophages have the ability to change their polarization in response to different stimuli. For example, macrophage phenotype changes during tissue repair, switching from proinflammatory phenotype (M1-like) to an anti-inflammatory phenotype (M2-like) . In addition, different polarization and activation markers of M1- and M2-like macrophages can coexist in tissues.

For instance, a high percentage of circulating macrophages expressing both M1 (CD80, CD86, and TLR4) and M2 surface markers (CD204, CD163, and CD206) was shown in human patients with interstitial lung disease .

With the analysis of the transcriptomic profiles of macrophages, Liu et al. reported that polarized M1- or M2-like macrophages driven by cytokines can be subsequently repolarized to

another phenotype with little or no memory of polarization history. Therefore, the in vivo phenotype and function of macrophages remains to be defined under specific tissue microenvironments. Phenotypic change and functional polarization of macrophages are accompanied by a change in cellular metabolism, as M1-like macrophages primarily rely on glycolysis, whereas M2-like macrophages rely on oxidative phosphorylation . Parallel analysis of macrophage metabolic and transcriptional profiles also indicates that metabolic reprogramming impacts macrophage polarization or activation .

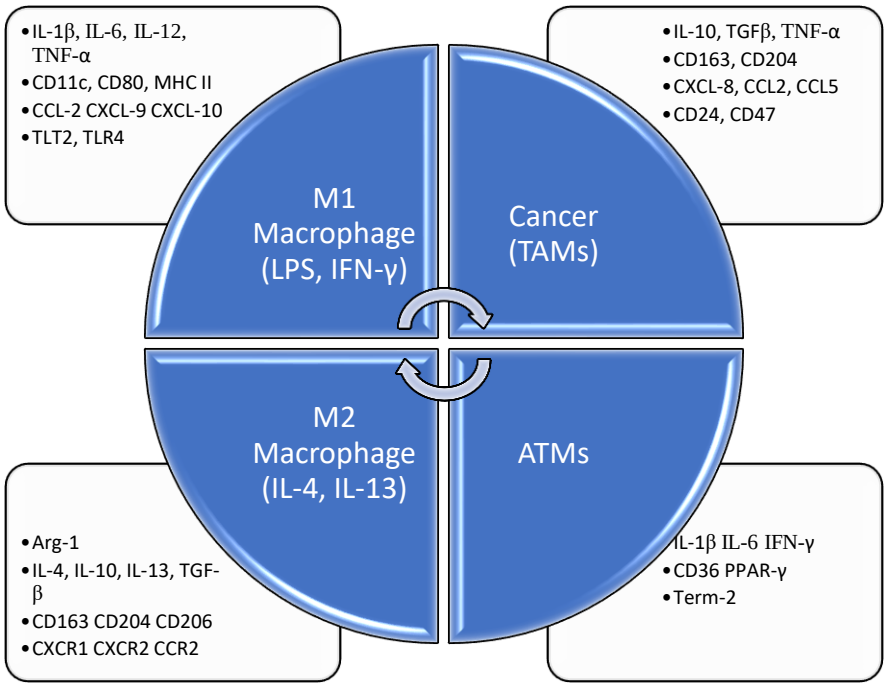
For example, inhibition of aspartateaminotransferase and N-glycosylation interfere with M1 and M2 macrophage polarization, respectively. Factors that affect macrophage metabolism may disrupt M1/M2 homeostasis. ...

multiple distinct populations of adipose tissue-associated macrophages (ATMs) are present in adipose tissues in mice and humans, existing with unique transcriptomes, chromatin landscapes, and functions. Similarly, pro-inflammatory ATMs in obese mice or humans can express additional markers of metabolic activation distinct from the classical markers of activation . In addition to the abilities of engulfing and digesting foreign pathogens and cellular debris, macrophages can clear away tumor cells.

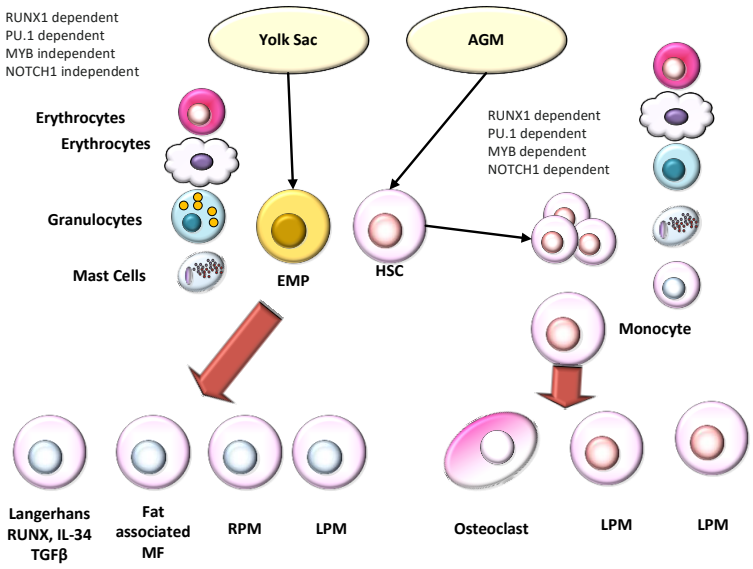
Tumor-associated macrophages (TAMs) are particularly abundant immune cells in cancer and exert strong influences on tumor initiation, progression, and metastasis . Besides, TAMs can secrete different cytokines such as IL-10 and transforming growth factor-β (TGF-β) to suppress T cell dependent antitumor function . TAMs can be polarized into pro-inflammatory (M1-like) phenotype and anti-inflammatory (M2-like) phenotype under the stimuli of different tumor microenvironments, with the majority of TAMs functioning as M2-like macrophages .

Notably, differentially polarized TAMs may exert opposite effects on tumor development. For example, high numbers of infiltrating M2-like macrophages in patients with gastric cancer (GC) were associated with a low rate of overall survival (OS), while an elevated number of M1-like macrophages was associated with better OS . Using scRNA-seq data and cell trajectory analysis, Landry et al. reported that core TAMs evolve toward a proinflammatory state in human glioblastoma, while peripheral TAMs develop an anti-inflammatory phenotype. Therefore, an accurate understanding of the function of TAMs is likely to advance cancer immunotherapy.

We summarize these in the following graphic:



Also see Recalcati et al for a discussion on metabolic factors of macrophages. Likewise for the work of Liu et al. Although not critical in our current analysis it does bring to the fore key biochemical factors. Similarly a good discussion is in Guo et al. as well as that of Kelly and O'Neil.



As Palma et al note the variety of M2 macrophages:

Macrophages derived from monocyte precursors undergo specific polarization processes which are influenced by the local tissue environment: classically activated (M1) macrophages, with a pro-inflammatory activity and a role of effector cells in Th1 cellular immune responses, and alternatively activated (M2) macrophages, with anti-inflammatory functions and involved in immunosuppression and tissue repair.

At least three different subsets of M2 macrophages, namely, M2a, M2b, and M2c, are characterized in the literature based on their eliciting signals.

The activation and polarization of macrophages is achieved through many, often intertwined, signaling pathways. To describe the logical relationships among the genes involved in macrophage polarization, we used a computational modeling methodology, namely, logical (Boolean) modeling of gene regulation.

We integrated experimental data and knowledge available in the literature to construct a logical network model for the gene regulation driving macrophage polarization to the M1, M2a, M2b, and M2c phenotypes. Using the software GINsim and BoolNet, we analyzed the network dynamics under different conditions and perturbations to understand how they affect cell polarization. Dynamic simulations of the network model, enacting the most relevant biological conditions, showed coherence with the observed behavior of in vivo macrophages.

The model could correctly reproduce the polarization toward the four main phenotypes as well as to several hybrid phenotypes, which are known to be experimentally associated to physiological and pathological conditions. We surmise that shifts among different phenotypes in the model mimic the hypothetical continuum of macrophage polarization, with M1 and M2 being the extremes of an uninterrupted sequence of states. …

Macrophages and neutrophils of the innate immune system represent the first line of defense against most common microorganisms.

Indeed, macrophages can recognize and respond to a wide range of stimuli, expressing a great variety of surface and intracellular receptors that activate several signal transduction pathways and complex gene expression patterns.

Macrophages respond to extracellular stimuli upon contact with different cell types via endocytic, phagocytic, and secretory functions.

Macrophages are quite useful and powerful cells. Thus attempting to use them in cancer therapeutics has great value. This analysis is a strong basis for the macrophage selection in reprogramming results.

Their activity is modulated by contact synapsis established with proximal cellular and molecular entities, including microorganisms, chemical mediators, and other macrophages.

The monocyte–macrophage differentiation pathway is known to exhibit plasticity and diversity. Similar to the polarization process of helper T type 1 and 2 cells (Th1–Th2), two distinct

polarized forms of macrophages have been recognized in the past: the classically activated (M1) macrophage phenotype and the alternatively activated (M2) macrophage phenotype. Moreover, macrophages have also been observed in "M2-like" states, which share some features of both M1 and M2.

Indeed, recent studies support the view that fully polarized macrophages (M1 and M2) are the extremes of a continuum of macrophage polarization.

For example, various stimuli, such as immune complexes (IC) together with LPS or interleukin-1 beta (IL-1b), glucocorticoids, transforming growth factor-b (TGF-b), and interleukin-10 (IL-10), give rise to M2-like functional phenotypes that share properties with IL-4- or IL-13-activated macrophages [such as high expression of mannose receptor (MR) and IL-10, as well as TNFa, IL-1b, and IL-6].

Variations of the gene expression patterns corresponding to M1 or M2 are also found in vivo (e.g., in the placenta and embryo, and during helminthic infection, Listeria infection, obesity, and cancer). The M1 and M2 phenotypes correspond to cell activation states driven by cytokines, which are typically secreted by Th1, Th2, and T-regulatory cells, but also basophils, mast cells, B lymphocytes, and eosinophils. The M1 phenotype is polarized by single or a combination of Th1 cytokines and pro-inflammatory mediators, including granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-a, IL-6, IL-1b, IL-12, and various pathogen-associated molecules, such as lipopolysaccharide (LPS).

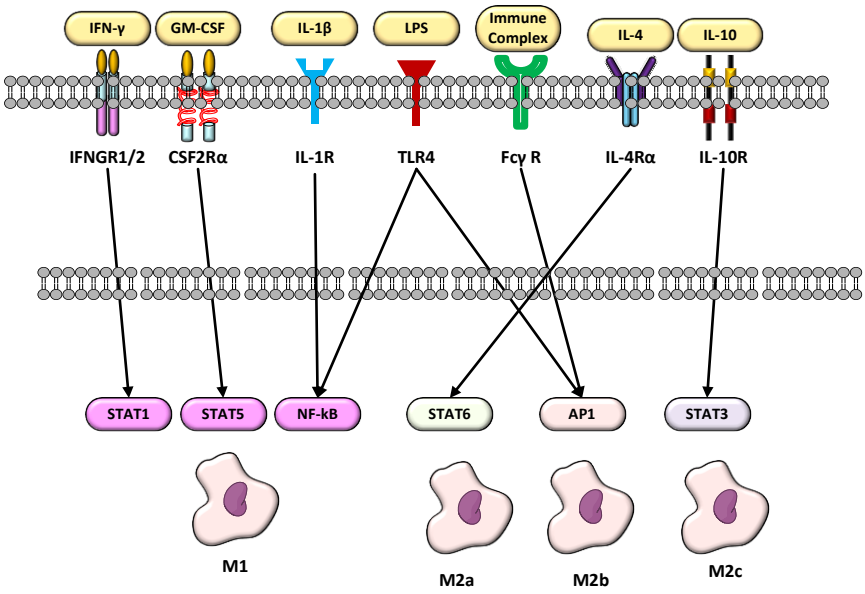
By contrast, the M2 polarization is induced by macrophage colony-stimulating factor (M-CSF), IL-4 and IL-13, IC, IL-10, as well as glucocorticoid, TGFb, and serotonin.

Although there is a wealth of information about the different macrophage subsets in vitro, features such as plasticity, heterogeneity, and adaptability make them very difficult to study using conventional experimental tools.

Furthermore, as many of the studies are done in different settings or for different goals, some literature reports are not conclusive and sometimes contradictory. It is not clear how robust the different macrophage subsets are to environmental changes. In particular, how does a modification of the cytokine environment affect the phenotype of macrophages? Which polarization state is most stable?

Which possible gene knockouts can lead to a phenotypic change? Macrophages polarization is essential in orchestrating the immune system response both in infectious and sterile immune settings. To shed light on this complex molecular process and address the questions above, we employed computational modeling of gene regulatory networks (GRNs).

We show the above results graphically below.



Macrophages are a diverse set of cells and play a diverse set of roles in the operation of the immune system. Thus when looking at macrophages we must understand this complexity.

2.3 PHAGOCYTOSIS AND MACROPHAGES

Macrophages as discussed above can stimulate other immune cells as well as destroy such cells by their own means. All of this discussion is essential in order to understand the selection of macrophages in the Patent. As Hirayama et al have noted:

Macrophages are effector cells of the innate immune system that phagocytose bacteria and secrete both pro-inflammatory and antimicrobial mediators.

In addition, macrophages play an important role in eliminating diseased and damaged cells through their programmed cell death.

Generally, macrophages ingest and degrade dead cells, debris, tumor cells, and foreign materials. They promote homeostasis by responding to internal and external changes within the body, not only as phagocytes, but also through trophic, regulatory, and repair functions. Recent studies demonstrated that macrophages differentiate from hematopoietic stem cell-derived monocytes and embryonic yolk sac macrophages. The latter mainly give rise to tissue macrophages.

Macrophages exist in all vertebrate tissues and have dual functions in host protection and tissue injury, which are maintained at a fine balance. Tissue macrophages have heterogeneous phenotypes in different tissue environments. …

Phagocytes such as neutrophils, macrophages, and dendritic cells make a bridge between specific bacterial surface antigens and cellular receptors. Following this bridge, membrane

protrusions surround the bacteria and absorb the bacteria into the phagosome, which is formed by the fusion f cell membranes.

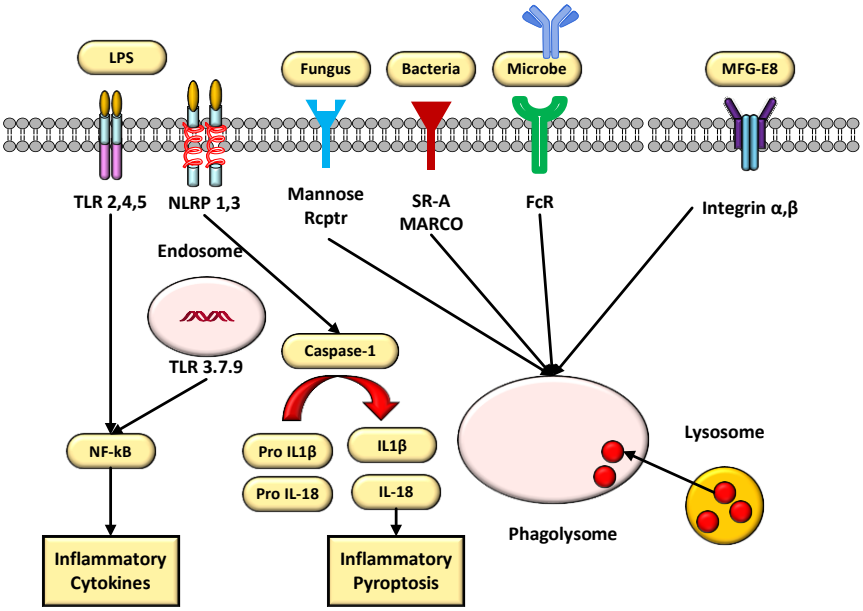
Since there is a wide range of phagocytic receptors, a variety of signaling cascades can be activated during this process. These receptors have various degrees of ligand specificity, and can be classified based on the type of ligands they recognize: foreign molecules identifiable by unique molecular patterns, opsonins, and apoptotic bodies. Phagocytes have several PRRs that bind specifically to certain PAMPs. For instance, the mannose receptor and Dectin-1 induce the phagocytosis of fungi with particular polysaccharides on their surface.

In addition, several scavenger receptors initiate phagocytosis upon PAMP recognition; these include the scavenger receptor A (SR-A) and the macrophage receptor with collagenous structure (MARCO), which bind to the surface molecules of Gram-negative and -positive bacteria.

Several soluble molecules, called opsonins, can be deposited onto foreign surfaces and serve as adaptors that bind and activate potent phagocytic receptors. For instance, immunoglobulin G (IgG), when specifically bound to microbial surface antigens, associates with fragment crystallizable γ receptors (FcγRs) in phagocytes, which recognizes their fragment crystallizable (Fc) region . The C3b and iC3b molecules of the complement system can also bind to foreign particles.

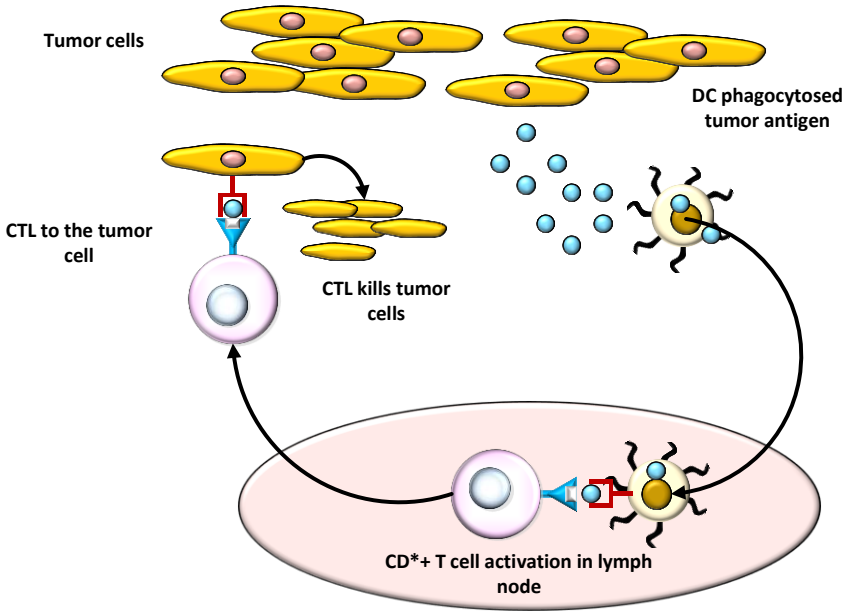
In addition to the clearance of foreign particles, phagocytosis is important for cell turnover within the organism, as billions of cells die by apoptosis every day, which must be removed. The mechanism of phagocytosis is different depending whether the cells are apoptotic or non-apoptotic. It is thought that the best-characterized signature of apoptotic cells is an increased surface exposure of the lipid phosphatidylserine (PS). In non-apoptotic cells, PS is mostly restricted to the inner leaflet of the plasma membrane. However, once the apoptosis pathway is triggered, the concentration of PS on the outside leaflet of the plasma membrane increases 300-fold. In addition, soluble proteins, such as milk-fat globular protein (MFG)-E8, growth arrest-specific protein (Gas)6, and protein S, bind to exposed PS and act as linkers, similar to opsonins

We demonstrate graphically some of these processes below:



2.4 MACROPHAGES AND CANCER

We now consider macrophages and cancer. The graphic below demonstrates the classic pathways of killing a cancer cell. It is simplistic, works at times, but fails often. The tumor cells provide Ag which in this case are taken up by dendrites. In the lymph nodes the DC activate T cells, creating cytotoxic T cells that go and kill the tumor cells. This works, sometimes! Not always.



Now as Abbas et al note:

Macrophages are capable of both inhibiting and promoting the growth and spread of cancers, depending on their activation state. Classically activated M1 macrophages, … can kill many types of tumor cells. How macrophages are activated by tumors is not known. A possible mechanism is recognition of damage-associated molecular patterns from dying tumor cells by macrophage innate immune receptors.

Macrophages in tumors also may be activated to kill tumor cells by IFN-γ produced by tumor-specific Th1 cells, CTLs, and NK cells.

This may be why a large number of Th1 cells in some tumors is correlated with a good prognosis.

M1 macrophages can kill tumor cells by mechanisms that they also use to kill infectious organisms, including the liberation of lysosomal enzymes, nitric oxide, and reactive oxygen species.

In the above we see the role of macrophages in this cancer killing process. As Ruffell and Coussens have noted:

Macrophages are represented in all tissues by functionally and phenotypically distinct resident populations that are critical for development and homeostasis. Under nonpathological conditions, most resident macrophage populations derive from embryonic progenitors and are maintained through local proliferation. Exceptions to this include intestinal, dermal, and alveolar macrophages at barrier sites and macrophages in the adult heart that are replaced by circulating bone marrow-derived Ly6C+ inflammatory monocytes over a timescale of several weeks. Under pathological conditions, there is evidence for both local proliferation and recruitment, with differences observed by tissue location and type of inflammatory insult.

Solid tumors appear to be unique. Preclinical studies indicate minimal macrophage proliferation and shorter half-lives compared with resident macrophages in counterpart homeostatic tissues, measurable in days to weeks.

That said, CD68+ cells also positive for proliferating cell nuclear antigen (PCNA) expression have been observed in breast cancers, where they are associated with a poor clinical outcome. Whether the macrophage lifespan in this context is reflecting diminished tissue integrity and the extent of damage/inflammation or, instead, represents an adaptive process engaged by tumors to support growth is unclear, but production of the C-C chemokine ligand 2 (CCL2) and/or colony-stimulating factor 1 (CSF-1) are necessary to sustain their numbers.

With the critical role for CCL2 and CSF-1 in recruiting macrophages to neoplastic tissue, there is growing interest in therapeutics targeting these ligands and/or their respective receptors in an effort to ablate the pro-tumorigenic properties of macrophages. This therapeutic approach has led to improved outcomes in a range of pre-clinical models, particularly for agents targeting CSF-1 or the CSF-1 receptor (CSF-1R), the results of which have spurred several clinical trials.

As monotherapy, CSF-1R inhibition alone impedes the growth of orthotopically implanted pancreatic ductal adenocarcinoma (PDAC) cell lines, prevents cervical carcinogenesis, and induces regression of glioblastoma multiforme (GBM). In other tumor models CSF-1R inhibition is without consequence as monotherapy.

However, synergism with other modalities, including chemotherapy, radiation therapy, angiogenic inhibitors, adoptive cell transfer, and immune checkpoint blockade have been revealed.

Together, these findings implicate macrophages in regulating therapeutic responses and indicate that durable responses may be obtained by augmenting standard of care or emerging therapies with ‘‘macrophage antagonists.’’

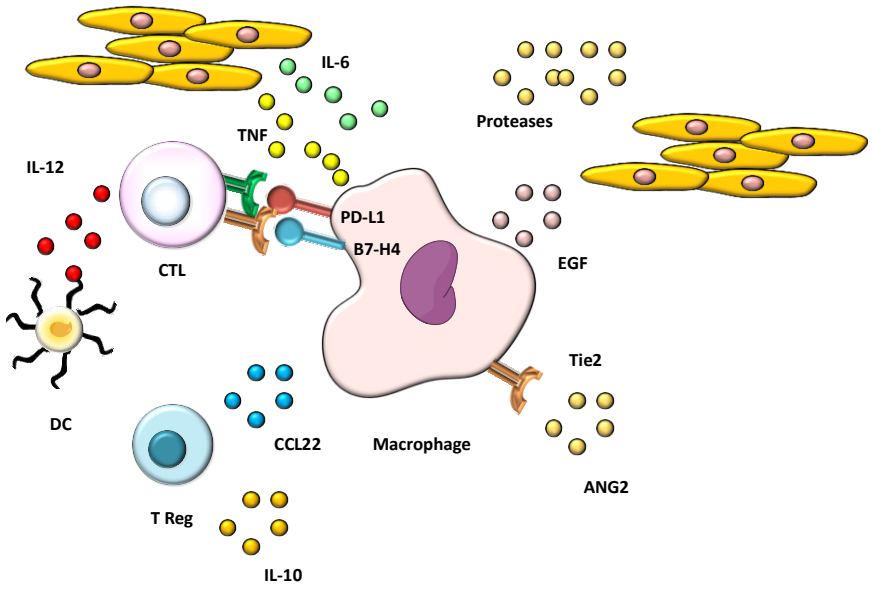
This review focuses on the mechanisms underpinning these observations and concludes with a discussion of targeting approaches that extend beyond inhibiting macrophage recruitment.

Clinical Significance of Macrophages *For many solid tumor types, high densities of cells expressing macrophage-associated markers have generally been found to be associated with a poor clinical outcome. There are conflicting data for lung, stomach, prostate, and bone, where both positive and negative outcome associations have been reported, possibly related to the type/stage of cancer evaluated, (e.g., Ewing sarcoma versus osteosarcoma) or to the type of analysis performed (e.g., quantitation of stromal versus intratumoral macrophages).*

Some discrepancy may also reflect the use of different macrophage markers. CD68, a glycoprotein predominantly resident in intracellular granules, represents a fairly specific marker for murine macrophages and, in combination with F4/80, identifies a majority of tumor-associated macrophages.

In humans, however, CD68 expression is widespread and includes granulocytes, dendritic cells, fibroblasts, endothelial cells, and some lymphoid subsets. The use of CD68 for association studies in this context is therefore of variable utility.

They summarize this in the following graphic:



The authors continue:

(A) Macrophage expression of IL-6 and TNF-a promotes survival signaling in neoplastic cells and resistance to chemotherapy and targeted agents. The expression of survival factors is dependent upon the protease activity of cathepsin B and/or S.

(B) Neoplastic cell invasion of ectopic tissue can be promoted through the directed release of cytokines/chemokines such as epidermal growth factor (EGF) and CCL18 or through protease-dependent extracellular matrix (ECM) remodeling that may directly affect neoplastic migration or increase chemoattractant bioavailability. EGF expression is driven by signaling through the CSF-1R via neoplastic cell production of CSF-1 as well as T cell-derived IL-4 (not shown).

(C) Macrophages directly promote angiogenesis via production of VEGFA and other angiogenic factors and can enhance VEGFA expression by endothelial cells through WNT7B. A subset of macrophages expressing the Tie2 receptor is recruited to the vasculature by mural cell/pericyte expression of ANG2 and is important in regulating vascular structure.

(D) Direct suppression of a cytotoxic T cell (CTL) response can occur via expression of B7 family ligands (PD-L1, B7-H4).

Indirect suppression may occur through release of IL-10 or recruitment of IL-10-expressing TRegs via CCL22, whereby IL-10 suppresses the capacity of dendritic cells to produce IL-12 and promote a TH1/CTL anti-tumor immune response.

2.5 Reprogramming Macrophages

We now consider the reprogramming of macrophages. We shall details this in the next chapter. As Bart et al have noted:

MΦ (macrophages) exhibit a high degree of plasticity in response to environmental signals, many of which are tissue- and context specific. This results in a variety of MΦ subtypes with different origins, which may play distinct roles in human disease and potentially provide unique opportunities for targeted therapies.

Before considering detailed examples of therapeutic approaches in specific tissue and disease contexts, we briefly introduce some common methods used to reprogram MΦ, from conventional approaches, such as targeted antibody treatments and small molecule drugs to cutting-edge technology of gene expression modification using viral vectors, artificial DNA carriers, naked DNA and cell therapy.

Approach	Pro	Con
Small molecule inhibitor and cytokines	Easy to administer	Short half-life and toxic
Nanovectors	Prolonged half-life and specific targeting	Toxicity
Viral Vectors (adenovirus, lentivirus)	Stability and enhanced targeting	Safety
Antibodies	High specificity and safe	Hish costs and systemic effects
Free Nucleic Acids	Easy to manufacture and low cost	Short half life

Summary of macrophage manipulation techniques for therapeutic purpose as shown above. These strategies can directly be applied in vivo, as well as in vitro followed by adaptive transfer of manipulated MΦ. Free nucleic acids can be manufactured easily and are very successfully used in some tissues including lungs and skeletal muscle. However, they lack MΦ specificity and are rapidly cleared from the environment, mostly by circulating enzymes and kidney. Viral vectors can be employed to deliver nucleic acids, preventing clearance from the system. Depending on the type of vector, gene manipulation can be long term (lentivirus) or transient (adenovirus).

Viral vectors are highly efficient and can be modified to improve MΦ targeting.

However, they do entail safety considerations for patients and manufacturing staff. Free small molecules and cytokines are known to act on MΦ polarization. They are easy to administer but prone to degradation. They are also often not MΦ specific and can cause off-target effects and toxicity. Encapsulation of nucleic acids, small molecules and cytokines into nanovectors prolongs their half-life in the organism, while surface modifications allow targeting of specific cell types. Antibodies can manipulate MΦ polarization by directly binding Fc or other cell surface receptors. While they are generally safe, high doses are often required for therapeutic efficacy translating into high costs.

They continue:

Targeted antibody treatments are among the easiest and most efficient methods to target not only MΦ surface receptors involved in the regulation of immune responses but also circulating cytokines/growth factors, preventing their interaction. As a result, antibodies can alter MΦ activation status. However, this technique is mostly systemic and can lead to numerous off-target effects. Gene therapy aims to alter specific gene expression by inserting genetic material into the target cell. Free nucleic acids can be directly injected in vivo.

While this is generally considered safe, detection by MΦ can induce inflammatory signalling. Although possibly advantageous when reprogramming MΦ into pro-inflammatory phenotypes, it may counter anti-inflammatory states and raises concern of off-target effects. Free nucleic acids also lack cell-targeting specificity, an issue that can be solved by attachment to carrier molecules, such as coupling to peptides directly targeting MΦ cell surface receptors. Nucleic acids can also be introduced using modified viral vectors that lack the genes necessary for replication.

Lentiviral vectors stably integrate genetic material into the host cell genome, while adenoviruses and adeno-associated viruses (AAVs) only cause transient gene expression.

Despite their high efficacy, viral vectors are associated with significant disadvantages: from a manufacturing perspective, viral vector production is costly and requires specific safety measurements.

From a clinical point of view, random lentiviral RNA insertion into the genome could cause tumour suppressor gene disruption triggering malignancy.

Also, viral vectors bear the risk of potentially high immunogenicity. Adenoviruses' triggering of immune responses could, however, be exploited for the use in tumour contexts where immune activation could be beneficial.

The latter observations are quite important. Sequellae from vaccines have been a critical factor in abandoning them. Adenoviruses seem to be a bit more benign in this case.

2.6 Cell Differentiation

It is helpful to understand the current models for hematopoietic cell differentiation. As Laiosa et al note:

The stochastic model of hematopoiesis states that a single multipotent progenitor (MPP) has the option to differentiate along more than two pathways. This early model was based on the observation that colony assays, using single myeloid progenitors, yielded highly heterogeneous outcomes.

As discussed below, most current models imply that hematopoietic differentiation proceeds along an ordered pathway with binary decision steps. However, ordered binary choices are not apparent, at least during the earliest stages of differentiation. The Akashi-Kondo-Weissman Scheme of Hematopoietic Differentiation The identification of stem and progenitor cells by

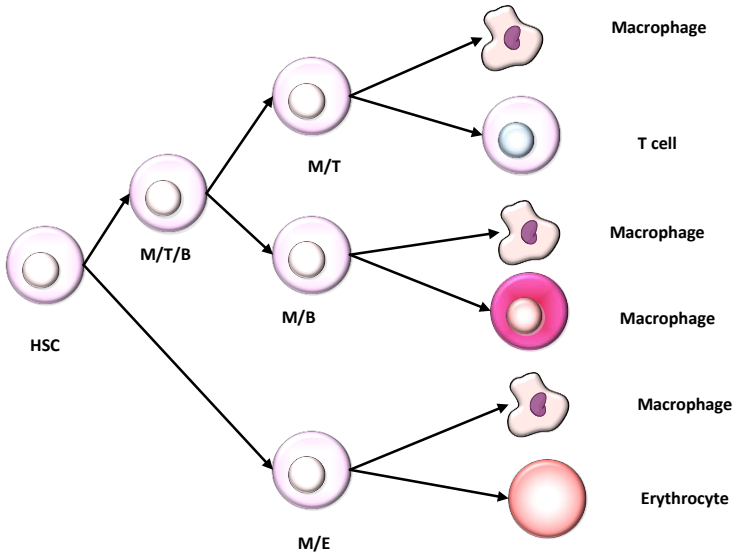
Weissman and collaborators led to the construction of a hematopoietic lineage tree that is characterized by a cascade of binary decisions. The staining of bone marrow, with a combination of cell surface antigenspecific antibodies led to the prospective isolation of hematopoietic stem cells (HSCs) as lin−/lowSca-1+c-kit+ (LSK) cells.

These cells can be further subdivided into long-term HSCs (Thy-1lowFlk2/Flt3−), short-term repopulating HSCs (Thy- 1lowFlt3+), and MPPs (Thy-1−Flt3+), populations that were also defined by other combinations of markers. Similar approaches led to the identification of progenitors with a more restricted differentiation potential.

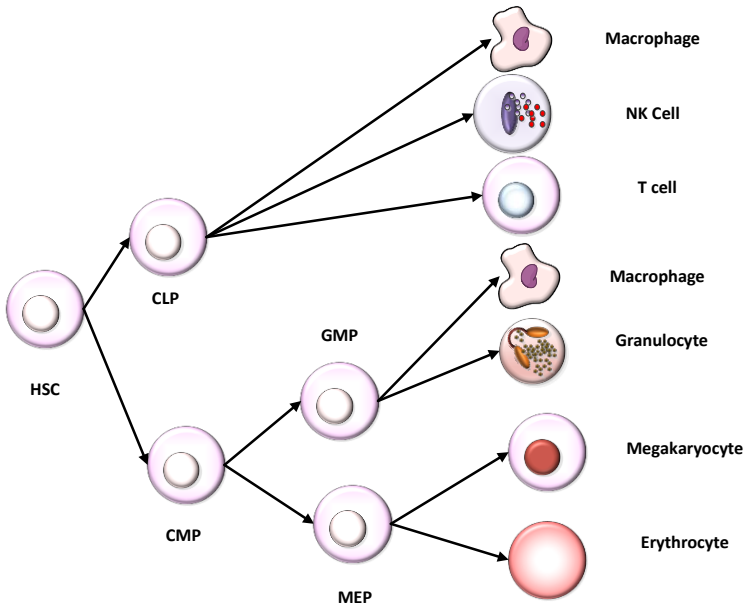
Thus, in the bone marrow investigators identified a common progenitor for all lymphoid lineages (CLP), as well as a common myeloid progenitor (CMP) that generates granulocytic-macrophage (GM) and megakaryocytic-erythroid (MegE) lineages. CLPs give rise to pro-B and pro-T cells, uncommitted lymphoid progenitors that will differentiate further into mature B and T cells. CLPs also produce NK lineage cells.

CMPs in turn generate two more restricted progenitors: granulocyte-macrophage progenitors (GMPs) and megakaryocyte-erythrocyte progenitors (MEPs), generating GM and MegE cells, respectively. The offspring of GMPs also includes neutrophils, eosinophils, and possibly basophils/mast cells. The observation that CMPs and CLPs derived from adult bone marrow generate mutually exclusive progeny suggests that their diversification represents the earliest branching point during hematopoietic differentiation.

The two structures for such differentiation are shown below:



And the second:



As Laiosa et al note:

Many macrophage- and granulocyte-restricted promoters are regulated by PU.1 and/or C/EBPα. These factors cooperate in the regulation of the genes encoding the myeloid growth factor receptors MCSFR, G-CSFR, and GM-CSFR.

In PU.1-deficient mice, all myelomonocytic cells are absent. However, as in the B cell lineage, PU.1 is not strictly required for commitment because immature myeloid precursor lines dependent on IL-3 can be established from PU.1-deficient fetal liver.

Conditional deletion of PU.1 in adult bone marrow, using the Mx1-Cre deleter system, leads to a complete loss of CMPs and GMPs.

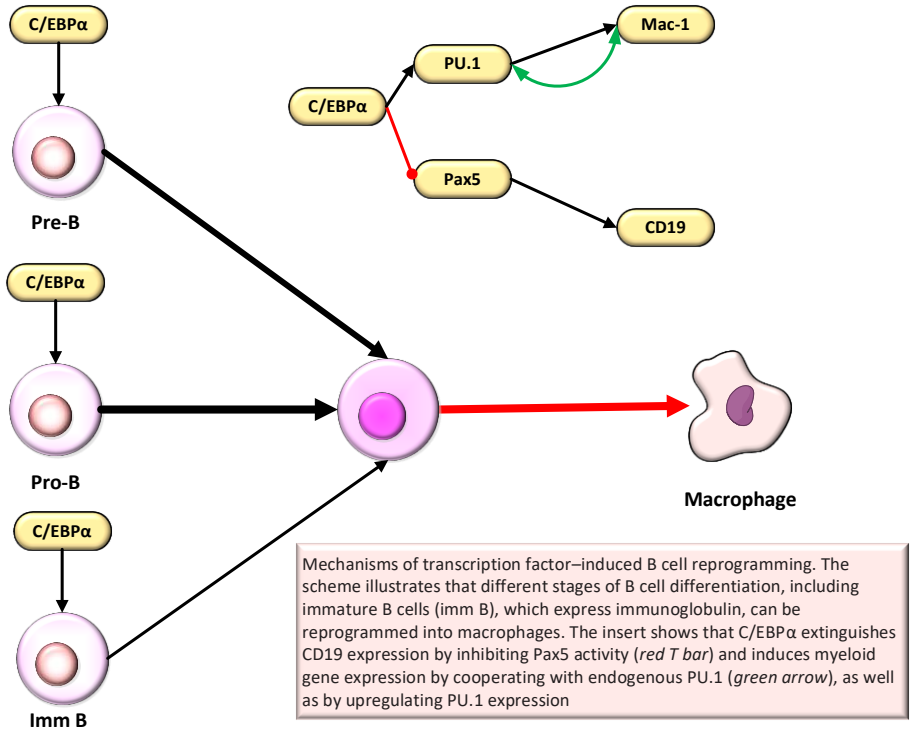
Mice deficient in C/EBPα lack neutrophil and eosinophil granulocytes, and conditional inactivation in the bone marrow shows the specific absence of GMPs and reduced numbers of CMPs, leading to decreased formation of all downstream lineages.

In colony assays, the remaining CMPs yielded normal MegE colonies and GM colonies containing cells with an immature phenotype. Deletion of C/EBPα by retroviral expression of Cre recombinase in GMPs does not prevent their terminal differentiation, showing that this factor is not needed at late stages, perhaps because its function is replaced by that of other C/EBP family members.

The functions of C/EBPα in granulocyte formation appear to be redundant with its close relative C/EBPβ because C/EBPβ expressed from the C/EBPα locus rescues neutrophil granulocyte development.

Macrophages from C/EBPβ-deficient mice develop normally but display defective bacterial cell killing. Unlike C/EBPα, C/EBPβ is also expressed in B cells, and C/EBPβ-deficient mice display defects in B cell expansion.

We depict this in the graphic below:

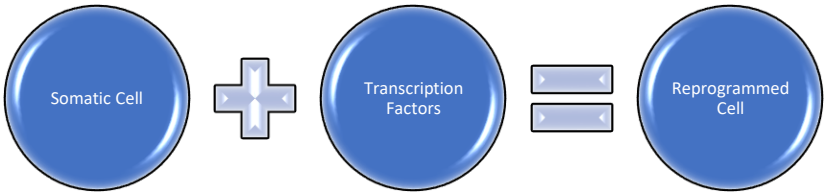


The above graphic is critical for understanding the generation of stem cells. Two gene products take us from a slightly committed cell to a committed cell. What we shall show latter is that this transition can take a more committed cell, a fibroblast., and perform the same transition. They continue:

The scheme illustrates that different stages of B cell differentiation, including immature B cells (imm B), which express immunoglobulin, can be reprogrammed into macrophages. The insert shows that C/EBPα extinguishes CD19 expression by inhibiting Pax5 activity (red T bar) and induces myeloid gene expression by cooperating with endogenous PU.1 (green arrow), as well as by upregulating PU.1 expression.

3 REPROGRAMMING

Reprogramming is in a sense a generalized term which reflects the change of a cell from one type to another. On of the most significant ones is the Nobel Prize winning work of Yamanata where he was able to take a somatic cell and convert it to an embryonic pluripotent stem cell, namely a cell which can then adopt other features as it matures. We examine this work briefly than return to the Majeti work where they take cancer cells and reprogram them to be antigen presenting cells, namely macrophages.



3.1 THE SEMINAL EXPERIMENT

Pluripotent cells have the ability to divide into a wide variety of mature cell types[3]. The classic paper was by Takahashi and Yamanaka in 2006. In that paper they note:

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions.

Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development.

These data demonstrate that pluripotent stem cells can be addition of only a few defined factors directly generated from fibroblast cultures …

[3] Pluripotent implies an ability to divide to a wide set of mature cells. Totipotent means that division cane proceed to reproduce the complete organism. Multipotent means a more limited maturation. Plant cells are generally considered pluripotent. See Verdeil et al, Pluripotent versus totipotent plant stem cells: dependence versus autonomy?, Trends in Plant Science, June 2007.

The above shows that a simple formula set of ingredients makes this dramatic change. The factors were gene products that we saw were critical for HSC development. From Yamanaka in his Nobel Prize Presentation we have a more details discussion of the process:

The first gene we knocked out in mice at the NAIST was ECAT3, also known as Fbox15. These mice were part of Yoshimi Tokuzawa's project. Yoshimi, Tomoko and I were very happy when we obtained the first targeted ES line, the first chimeric mice and then germ-line transmission. However, when we generated homozygous mutant mice lacking the functional ECAT3 gene, we did not observe any obvious phenotypes . Because of its specific expression in mouse ES cells and embryos, we expected that its disruption would result in early lethality during embryogenesis.

Furthermore, we showed that Fbox15 is a direct target of Oct3/4 and Sox2, another transcription factor essential for the maintenance of pluripotency.

On the contrary, we obtained homozygous mutant mice in accordance with the Mendelian law from heterozygous intercrosses. Yoshimi then generated homozygous mutant ES cell lines, hoping that she would observe drastic phenotypes. However, again, we did not see any significant changes. ECAT3-null ES cells proliferated normally and showed normal differentiation potentials. Thus, both the ECAT3 knockout mice and ES cells were apparently normal. This often happened with other ECATs. These experiences reminded us that science is often tough.

An exception was ECAT4, a transcription factor that was later re-named Nanog. We and others found that Nanog played important roles in the maintenance of pluripotency in mouse ES cell. Nanog was also essential for mouse embryonic development before implantation. In addition to Nanog, Yoshimi Tokuzawa identified another transcription factor, Klf4 that played important roles in mouse ES cells. Another group reported the important role of the well-known oncogene, c-Myc, in pluripotency.

By 2004, we had identified a total of 24 factors, including Oct3/4, Sox2, c-Myc, Nanog, other ECATs and Klf4, as candidate reprogramming factors. What we then needed was a sensitive and rapid assay system to screen these candidate factors. It turned out that the Fbox15-null knockout mice provided such an assay system. When we made knockout mice of Fbox-15 and other ECATs, we utilized a gene trap strategy, in which we knocked the neomycin resistance gene into the gene of interest. Thus, in ECAT3 knockout cells, the neomycin resistance gene is expressed from the enhancer and promoter of ECAT3, which was active only in ES cells and early embryos, but not in somatic cells.

Somatic cells, such as mouse embryonic fibroblasts (MEFs) derived from the ECAT3 knockout mice are sensitive to G418, whereas ECAT3 knockout ES cells were resistant to high concentrations of G418.

Based on these results, we expected that if any of the 24 candidates could actually induce pluripotency in ECAT3 knockout MEFs, the reprogrammed cells would become resistant to G418.

We confirmed this strategy by using a fusion reprogramming system. The ECAT3 knockout mice that showed few phenotypes and thus disappointed Yoshimi and me turned out to provide a very useful assay system to evaluate candidate reprogramming factors. My lab moved to Kyoto University in 2005, with the 24 gene candidates, the ECAT3-based assay system and Tomoko Ichisaka and Kazutoshi Takahashi. In Kyoto, I asked Kazutoshi Takahashi to examine the 24 factors by using the assay system

To tell the truth, we did not expect that we had the answer among these 24 factors.

We thought we had to screen many more factors, and had already started to prepare cDNA libraries from mouse ES cells and testes. Nevertheless, Kazutoshi introduced each of the 24 candidate genes, one by one, into ECAT3 knockout MEFs by retroviral transduction. As, in a sense, expected, we did not obtain any drug-resistant colonies using any single factor, thus indicating that no single candidate gene was sufficient to elicit reprograming and induce pluripotency.

In addition to the single factor transduction, Kazutoshi proposed to transduce all 24 factors together into ECAT3 knockout MEFs as a practice for performing a cDNA library screening. It was like a mini-library consisting of 24 cDNAs. To our surprise, four weeks after transduction, we obtained several G418-resistant colonies. I thought this might be some kind of mistake, such as contamination with ES cells. I asked Kazuthoshi to repeat the experiment again and again. It always worked. Kazutoshi picked up the G418-resistant colonies for expansion. We found that these cells were expandable and showed a morphology similar to that of mouse ES cells.

A reverse transcription PCR (RT-PCR) analysis revealed that the iPS-MEF24 clones expressed ES cell markers, including ***Oct3/4, Nanog, E-Ras, Cripto, Dax1, Zfp296 and Fgf4*** *.*

Next, to determine which of the 24 candidates were critical, Kazutoshi examined the effects of withdrawal of individual factors from the pool of transduced candidate genes. ES cell-like colonies did not form when either Oct3/4 or Klf4 was removed.

The removal of Sox2 resulted in only a few ES-like colonies. When he removed c-Myc, the ES cell-like colonies did emerge, but these had a flatter, non-ES-cell-like morphology.

Removal of the remaining factors did not significantly affect the colony numbers or characteristics.

We finally showed that a combination of four genes, Oct3/4, Klf4, Sox2 and c-Myc was sufficient to produce ES cell-like colonies.

These data demonstrated that pluripotency could be induced from MEF culture by the introduction of four transcription factors; Oct3/4, Sox2, c-Myc and Klf4.

I designated the new pluripotent stem cells "iPS cells," short for induced pluripotent stem cells.

We examined the pluripotency of iPS cells by the teratoma formation assay in animals. We obtained tumors from iPS cells after subcutaneous injection into nude mice. A histological examination revealed that the iPS cells differentiated into all three germ layers, including neural tissues, cartilage and columnar epithelium. We also examined the ability of iPS cells to produce adult chimeras.

We injected iPS cells into mouse-derived blastocysts, which we then transplanted into the uteri of pseudo-pregnant mice. We obtained adult chimeras from those injected iPS cells as determined by the coat color of the resulting pups. From these chimeras, we were able to obtain F1 mice through germline transmission.

Based on these results, we concluded that iPS cells are comparable to * in terms of their pluripotency.

The following Table details some of the key genes:

Gene	Function (from NCBI)
Oct3/4	This gene encodes a transcription factor containing a POU homeodomain that plays a key role in embryonic development and stem cell pluripotency. Aberrant expression of this gene in adult tissues is associated with tumorigenesis. This gene can participate in a translocation with the Ewing's sarcoma gene on chromosome 21, which also leads to tumor formation. Alternative splicing, as well as usage of alternative AUG and non-AUG translation initiation codons, results in multiple isoforms. One of the AUG start codons is polymorphic in human populations. Related pseudogenes have been identified on chromosomes 1, 3, 8, 10, and 12.
Klf4	This gene encodes a protein that belongs to the Kruppel family of transcription factors. The encoded zinc finger protein is required for normal development of the barrier function of skin. The encoded protein is thought to control the G1-to-S transition of the cell cycle following DNA damage by mediating the tumor suppressor gene p53. Mice lacking this gene have a normal appearance but lose weight rapidly, and die shortly after birth due to fluid evaporation resulting from compromised epidermal barrier function. Alternative splicing results in multiple transcript variants encoding different isoforms.
Sox2	This intronless gene encodes a member of the SRY-related HMG-box (SOX) family of transcription factors involved in the regulation of embryonic development and in the determination of cell fate. The product of this gene is required for stem-cell maintenance in the central nervous system, and also regulates gene expression in the stomach. Mutations in this gene have been associated with optic nerve hypoplasia and with syndromic microphthalmia, a severe form of structural eye malformation. This gene lies within an intron of another gene called SOX2 overlapping transcript (SOX2OT).
c-Myc	This gene is a proto-oncogene and encodes a nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. The encoded protein forms a heterodimer with the related transcription factor MAX. This complex binds to the E box DNA consensus sequence and regulates the transcription of specific target genes. Amplification of this gene is frequently observed in numerous human cancers. Translocations involving this gene are associated with Burkitt lymphoma and multiple myeloma in human patients. There is evidence to show that translation initiates both from an upstream, in-frame non-AUG (CUG) and a downstream AUG start site, resulting in the production of two isoforms with distinct N-termini.

3.2 CANCER CELLS TO APCS

We now consider reprogramming of cancer cells to macrophages. From Majeti et al, Patent Filing of: *Lineage Reprogramming As A Cancer Immunotherapy World Intellectual Property Organization May 6, 2021.* **Henceforth called "the Patent***".*[4]

Compositions and methods are provided for vaccination with cancer cells. The cancer cells are treated by direct reprogramming to generate antigen presenting cells, which can present tumor antigens and enhance immune responsiveness to the cancer. The direct reprogramming utilizes genetic modification to force expression of transcription factors that drive cells into the myeloid lineage.

In some embodiments the transcription factors are selected from one or more of C/EBPa, PU.1, BATF3 and IRF8. In some embodiments the transcription factors comprise or consist of one or both of C/EBPa and PU.1.

It is surprisingly found that cancer cells from both hematologic cancers, such as leukemias and lymphomas, and non-hematologic cancers, such as carcinomas, fibrosarcomas, osteosarcomas, etc. can be reprogrammed to present antigen. The methods can be applied to cancer cell lines, and to primary cancer cells, including patient-derived cancer cells. [0009] Methods of direct reprogramming of cancer cells utilize introduction of genetic construct(s) encoding myeloid lineage transcription factors operably linked to a promoter that is active or can be activated in the cancer cell; or mRNA coding sequences. Reprogramming can be performed ex vivo, e.g. with patient-derived cancer cells, or in vivo.

Direct in vivo reprogramming of cancer cells in situ can utilize delivery of reprogramming factors by viral vectors, mRNA, etc., for example by introducing the reprogramming factors at the site of the tumor.

The myeloid lineage transcription factors may comprise or consist of one or both of C/EBPa and PU.1.

In some embodiments the cancer cells are transduced with genes encoding both C/EBPa and PU.1. In some embodiments the genetic construct is integrated into the cancer cell genome. In some embodiments the construct is a viral vector, e.g. a retroviral or lentiviral vector. The promoter may be constitutive or inducible, e.g. a tet-inducible promoter. Alternatively the cancer cell genome is edited, e.g. by CRISPR technology, to operably link the transcription factors to a constitutive or inducible promoter.

The genetically modified cancer cells, if cultured ex vivo, can be cultured in the presence of cytokines that enhance myeloid differentiation.

Cytokine culture conditions include, without limitation, one or more of Flt3-L, IL-7, IL-3, GM-CSF, m-CSF and IL-4. Combinations of cytokines may be provided. Following the expression of

[4] https://patents.google.com/patent/WO2021087234A1/en

the myeloid lineage transcription factors, the cancer cells are found to express cell-surface markers characteristic of myeloid, antigen-presenting cells. Such markers include, without limitation, CD11b, CD14, SIRPa, Ly6c and CD115.

In some embodiments the reprogrammed cancer cells are CD11b+ and CD14+.

In some embodiments, the reprogrammed cells are selected for expression of one or both of CD11b and CD14 prior to use as a vaccine, e.g. by flow cytometry or magnetic selection methods.

In some embodiments, T cells, usually autologous T cells, are stimulated ex vivo by contact with the reprogrammed cancer cells, and the T cells thus activated are re-introduced into the patient.

In some embodiments a therapeutic method is provided, the method comprising introducing into a recipient in need thereof, e.g. a cancer patient, an effective dose of a reprogrammed cancer cell population, wherein the cell population comprises cancer cells that have been genetically modified to force expression of transcription factors that drive cells into the myeloid lineage. In some embodiments the transcription factors are one or both of C/EBPa and PU.1.

The initial cell population may be reprogrammed ex vivo, and is usually autologous or allogeneic with respect to the recipient. The starting cell population may be derived from the patient to be treated. The reprogrammed cancer cell population may be selected prior to administration for expression of one or both of CD11b and CD14. The dose of reprogrammed cancer cells is effective in increasing immune responses against the cancer.

3.2.1 Lineage

The Patent further states:

The data … indicate that ectopic CEBPa and PU.1 expression efficiently reprograms a diverse selection of hematologic and solid tumor cells into myeloid-lineage, macrophage-like cells*. This is the first evidence that solid tumor models can be forced to undergo lineage reprogramming via ectopic expression of transcription factors into cells that have characteristics of antigen-presenting cells*

3.2.2 Genes

The following Table summarizes some of the principal genes targeted.

Gene	Function (from NCBI)
C/EBPα	*This intronless gene encodes a transcription factor that contains a basic leucine zipper (bZIP) domain and recognizes the CCAAT motif in the promoters of target genes.* *The encoded protein functions in homodimers and also heterodimers with CCAAT/enhancer-binding proteins beta and gamma. Activity of this protein can modulate the expression of genes involved in cell cycle regulation as well as in body weight homeostasis.* *Mutation of this gene is associated with **acute myeloid leukemia**. The use of alternative in-frame non-AUG (GUG) and AUG start codons results in protein isoforms with different lengths. Differential translation initiation is mediated by an out-of-frame, upstream open reading frame which is located between the GUG and the first AUG start codons.*
PU.1	*This gene encodes an ETS-domain transcription factor that activates gene expression during myeloid and B-lymphoid cell development.* *The nuclear protein binds to a purine-rich sequence known as the PU-box found near the promoters of target genes, and regulates their expression in coordination with other transcription factors and cofactors. The protein can also **regulate alternative splicing of target genes**.*
BATF3	*This gene encodes a member of the basic leucine zipper protein family. The encoded protein functions as a transcriptional repressor when heterodimerizing with JUN. The protein may play a role in repression of interleukin-2 and matrix metalloproteinase-1 transcription*
IRF8	*Interferon consensus sequence-binding protein (ICSBP) is a transcription factor of the interferon (IFN) regulatory factor (IRF) family. Proteins of this family are composed of a conserved DNA-binding domain in the N-terminal region and a divergent C-terminal region that serves as the regulatory domain. The IRF family proteins bind to the IFN-stimulated response element (ISRE) and regulate expression of genes stimulated by type I IFNs, namely IFN-alpha and IFN-beta. IRF family proteins also control expression of IFN-alpha and IFN-beta-regulated genes that are induced by viral infection.*

3.2.3 Preparation

From the Patent they note the implementation as follows:

In the present methods, myeloid reprogramming factors are ectopically expressed in cancer cells by introducing an expression vector into the cells, or by genome editing the cells to operably link the transcription factors to a promoter of interest.

The transcription factor coding sequences may be introduced on an expression vector into the cell to be reprogrammed. DNA encoding the transcription factors may be obtained from various sources as desired during the engineering process.

The nucleic acid encoding the transcription factors is inserted into a replicable vector for expression.

Many such vectors are available. The vector components generally include, but are not limited to, one or more of the following: an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Vectors include viral vectors, plasmid vectors, integrating vectors, and the like. Lentiviral vectors are of particular interest. Other viral vectors of interest include adenovirus vectors, AAV vectors, and the like.

Expression vectors usually contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium.

Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media.

Expression vectors will contain a promoter that is recognized by the host organism and is operably linked to the transcription factor coding sequences.

Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence to which they are operably linked.

Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature.

A large number of promoters recognized by a variety of potential host cells are well known.

Transcription from vectors in mammalian host cells may be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus (such as murine stem cell virus), hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin

promoter, PGK (phosphoglycerate kinase), or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication.

3.2.4 Introduction of Cells

Generally at least about 10^4 reprogrammed cells/kg are administered, at least about 10^5 reprogrammed cells /kg; at least about 10^6 reprogrammed cells /kg, at least about 10^7 reprogrammed cells/kg, at least about 10^8 reprogrammed cells/kg, at least $1x10^9$ cells/kg, at least $1x10^{10}$ cells/kg, or more.

For example, typical ranges for the administration of cells for use in the practice of the present invention range from about $1x10^5$ to $5x10^8$ viable cells per kg of subject body weight per course of therapy.

Consequently, adjusted for body weight, typical ranges for the administration of viable cells in human subjects ranges from approximately $1x10^6$ to approximately $1x10^{13}$ viable cells… A course of therapy may be a single dose or in multiple doses over a period of time.

4 CANCER THERAPUTICS

We now will consider a broader base of cancer therapeutics albeit those aligned with what we have been considering with reprogramming.

4.1 REPROGRAMMING AND CANCER

We start with reprogramming approaches. As Gong et al noted:

In the past decade, remarkable progress has been made in reprogramming terminally differentiated somatic cells and cancer cells into induced pluripotent cells and cancer cells with benign phenotypes. Recent studies have explored various approaches to induce reprogramming from one cell type to another, including lineage-specific transcription factors-, combinatorial small molecules-, microRNAs- and embryonic microenvironment-derived exosome-mediated reprogramming.

These reprogramming approaches have been proven to be technically feasible and versatile to enable re-activation of sequestered epigenetic regions, thus driving fate decisions of differentiated cells. One of the significant utilities of cancer cell reprogramming is the therapeutic potential of retrieving normal cell functions from various malignancies. However, there are several major obstacles to overcome in cancer cell reprogramming before clinical translation, including characterization of reprogramming mechanisms, improvement of reprogramming efficiency and safety, and development of delivery methods.

Recently, several insights in reprogramming mechanism have been proposed, and determining progress has been achieved to promote reprogramming efficiency and feasibility, allowing it to emerge as a promising therapy against cancer in the near future. This review aims to discuss recent applications in cancer cell reprogramming, with a focus on the clinical significance and limitations of different reprogramming approaches, while summarizing vital roles played by transcription factors, small molecules, microRNAs and exosomes during the reprogramming process

4.2 CANCER VACCINES

Vaccines have been used extensively in many areas and cancer vaccines have been examined as well with less than satisfactory results. As Saxena et al note:

Cancer vaccines typically involve exogenous administration of selected tumour antigens combined with adjuvants that activate DCs, or even DCs themselves.

The aim of therapeutic cancer vaccines is to stimulate the patient's adaptive immune system against specific tumour antigens to regain control over tumour growth, induce regression of established tumours and eradicate minimal residual disease.

The basic principles needed for successful therapeutic vaccination against tumours include delivery of large amounts of a high-quality antigen to DCs, optimal DC activation, induction of strong and sustained CD4+ T helper cell and cytotoxic T lymphocyte (CTL) responses, infiltration of the TME and durability and maintenance of response.

This may be accomplished by several methods, such as reversal of tumour-induced immune exhaustion by immune checkpoint inhibitors, activation of DCs and effector T cells by administration of tumour-associated antigens with adjuvants or vaccination with autologous DCs loaded with specific tumour antigens.

Alternatively, the tumour's local immune environment can be broadly activated to induce tumour cell death, and it is possible to facilitate tumour antigen availability by use of in situ vaccines (ISVs). As opposed to traditional vaccines, where antigens are carefully selected, purified or prepared and injected into patients, the in situ approach generates the vaccine in the TME itself by sourcing the antigens from dead or dying tumour cells.

We have included ISVs in our profile of cancer vaccines as these fulfil the basic requirements of vaccines; that is, delivery of antigens to tumour-infiltrating DCs to provoke an adaptive T cell response. Despite the FDA approval of a DC-focused cell-based vaccine, sipuleucel-T, more than 10 years ago, no other therapeutic cancer vaccine has been approved. It is now appreciated that tumour cell intrinsic resistance and local or systemic immunosuppressive (extrinsic) mechanisms substantially compromise the efficacy of cancer vaccines.

The implementation of immunotherapies such as immune checkpoint inhibition (ICI) — for example, anti-CTLA-4, anti-PD1 and anti-PDL1 antibodies — to overcome resistance has effectively changed cancer care, substantially increasing response rates and even leading to potential cures. Therapeutic cancer vaccines are re-emerging as approaches to increase response rates and survival, especially in combination with ICI …

The authors continue:

Tumour immunity regulation. Immune cells are driven to the tumour microenvironment (TME) via a chemokine gradient. In the TME, dendritic cells (DCs) take up and process tumour antigens and present them on MHC class II or MHC class I molecules (through cross-presentation) (bottom left).

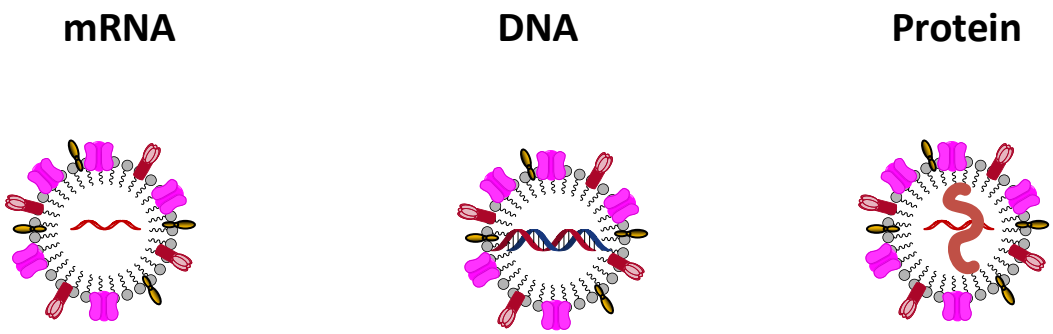
Cross-presentation may occur through the cytosolic pathway or the vacuolar pathway. In the cytosolic pathway, antigens from endosomes or phagosomes are transferred into the cytosol, proteasomally cleaved and transported to the endoplasmic reticulum (ER). Next, peptides are further edited, loaded on MHC class I molecules and transported to the cell surface.

Conversely, after cytosolic proteolytic cleavage, shortened peptides may be transferred back to phagosomes, loaded on MHC class I molecules and transported to the cell surface. In the vacuolar pathway, antigens are processed and loaded onto MHC class I molecules in the phagosomes or endosomes. Antigen-loaded DCs travel to the secondary lymphoid organ (SLO) and activate T cells…

Interaction between MHC–peptide complex–T cell receptor (TCR) and cognate receptor–ligand pairs induces DCs to secrete cytokines and activate T cells. CD8+ T cell responses are amplified by IL-2 secreted by CD4+ T cells. Activated T cells travel to the TME and induce tumour killing. Tertiary lymphoid structures (TLS) (top right) often develop in the TME266. Here, antigen-loaded DCs activate T cells and follicular DCs (fDCs) facilitate the generation of memory B cells and antibody producing plasma cells.

Activated T cells, B cells and antitumour antibodies facilitate tumour cell death by direct tumour cell lysis, antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC)[5].

The three major types of vaccines are shown below:



The authors note:

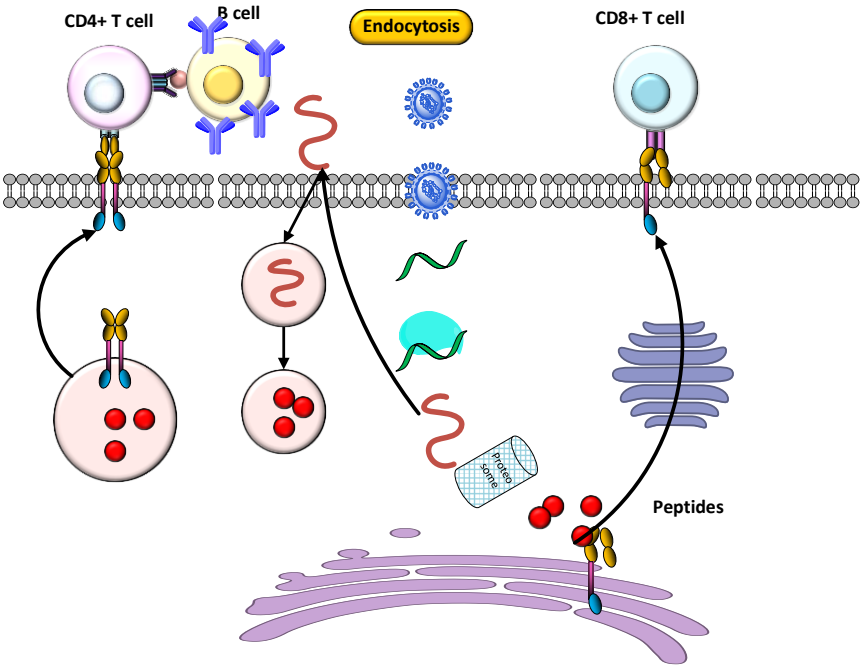
Antigens, either shared antigens or neoantigens, can be delivered directly via antigen-based cancer vaccines, formulated with the desired adjuvant and administered directly into the patient subcutaneously, intramuscularly, intra-nodally (in the lymph nodes) or intravenously. DNA vaccines, compared with RNA vaccines, require more processing steps before being presented on dendritic cells (DCs), while peptide vaccines have the shortest processing route. However, DNA and RNA vaccines are more suited as compared with peptide vaccines to deliver the antigen for MHC class I presentation.

Moreover, DNA vaccines can be electroporated directly at the injection site, while RNA vaccines maybe delivered intravenously with newly developed nanoparticles such as lipoplexes, which facilitate delivery of the vaccine to lymph node-resident DCs.

4.2.1 mRNA Vaccines

[5] ECM, extracellular matrix; ERAP1, endoplasmic reticulum aminopeptidase 1; FLT3L, fms-like tyrosine kinase 3 ligand; NK, natural killer; TAA, tumour-associated antigen; TAP; transporter associated with antigen Processing

mRNA has become a viable vaccine process as has been seen in the use of COVID. The overall process can be shown below which is a modification of what we have examined in COVID.



As Lorentzen et al had noted:

The number of clinical trials with therapeutic mRNA cancer vaccines is rapidly expanding, taking advantage of recent research advances that have optimised mRNA delivery, simplified administration methods, and improved translational efficiency. Despite substantial progress, several challenges to mRNA vaccine immunogenicity and efficacy remain. Thus, one of the most important advances in therapeutic clinical cancer vaccines is the ability to identify individual cancer neoantigens.

However, identifying tumour-specific mutations or non-conforming sequences and predicting corresponding neoepitopes for individual HLA alleles remains difficult. Furthermore, the technological and regulatory hurdles that will arise from the need for rapid and large-scale good manufacturing practice production of individualized mRNA vaccines are future obstacles that will need to be addressed. Another challenge is to validate the most feasible vaccine administration methods.

The administration route determines mRNA distribution and influences vaccine efficacy. mRNA that is injected intradermally and subcutaneously is easily processed by regional antigenpresenting cells, but the administrations often induce considerable local injection-site reactions. Intranasal administered mRNA reaches antigen-presenting cells in the peripheral

lymph nodes, and intranodal injections reach lymphatic antigen-presenting cells directly, but the delivery methods are cumbersome and only allow for small injection volumes.

The same limitations apply to intratumoural injections, and this administration route primarily aims to induce local inflammation with mRNA encoding co-activating molecules. Muscle tissue is highly vascularised, contains diverse immune cells for mRNA processing, and intramuscular injection induces fewer injection-site reactions in general.

Intramuscular administration is, therefore, a common and feasible vaccination route, and the current approved mRNA SARS-CoV-2 vaccines are administered intramuscularly. Intravenous injections allow mRNA to reach numerous lymphoid organs, and this administration method has been shown to induce a robust CD8+ T-cell response compared with local injections. CD8+ T cells have a central role in anti-tumour responses, and intravenous injection is the most common direct administration route in active therapeutic mRNA cancer vaccine trials. Most mRNA-based cancer vaccines are therapeutic rather than prophylactic, and require multiple administrations and substantial vaccine potency to induce a tumour response when given as monotherapy.

Monotherapy mRNA-based vaccines could be an effective treatment for patients diagnosed with early-stage cancer or in an adjuvant setting, but it appears unlikely that the vaccines will succeed as a monotherapy treatment for advanced cancers because of challenges regarding the highly immunosuppressive tumour microenvironment of this setting.

Therapeutic mRNA cancer vaccines are more likely to succeed in combination with other immunotherapeutic treatment methods such as immune checkpoint inhibitors, oncolytic viruses, and adoptive cell therapy. Indeed, patients receiving these combinations show encouraging clinical treatment responses across cancer diagnoses.

There is a need for new treatment combinations that increase response rates and progression-free survival without inducing severe side effects, and an mRNA cancer vaccine with low toxicity is an obvious combination partner.

Esprit et al have examined the Ag issues regarding mRNA vaccines:

The interest in therapeutic cancer vaccines has caught enormous attention in recent years due to several breakthroughs in cancer research, among which the finding that successful checkpoint blockade treatments reinvigorate neo-antigen-specific T cells and that successful adoptive cell therapies are directed towards neo-antigens. Neo-antigens are cancer-specific antigens, which develop from somatic mutations in the cancer cell genome that can be highly immunogenic and are not subjected to central tolerance.

As the majority of neo-antigens are unique to each patient's cancer, a vaccine technology that is flexible and potent is required to develop personalized neo-antigen vaccines. In vitro transcribed mRNA is such a technology platform and has been evaluated for delivery of neo-antigens to professional antigen-presenting cells both ex vivo and in vivo. In addition, strategies that support the activity of T cells in the tumor microenvironment have been developed. These represent a unique opportunity to ensure durable T cell activity upon vaccination. Here, we

comprehensively review recent progress in mRNA-based neo-antigen vaccines, summarizing critical milestones that made it possible to bring the promise of therapeutic cancer vaccines within reach. …

Neo-antigens can result from any change to the cancer genome that leads to the production of proteins with an altered protein sequence. These DNA changes can include non-synonymous mutations or single nucleotide variants (SNV), mutational frameshifts (insertions/deletions [INDELs]), gene fusions, post-translational modifications that alter the amino acid sequence and intron retention .

These neo-antigens are abnormal proteins that are subjected to cytosolic degradation regulated by ubiquitin and mediated by the proteasome. As a result, short peptides (2–20 amino acid residues) are generated, of which some are transported to the endoplasmic reticulum for loading onto MHC-I molecules . These peptide-loaded MHC-I molecules find their way through the Golgi apparatus to the cell membrane, resulting in antigen presentation and as a result the potential of recognition by the T cell receptor (TCR) of CD8+ T cells, which can then be activated to CTLs .

However, spontaneous activation of CTLs to neo-antigen derived epitopes (neo-epitopes) is not a frequent event . Neo-epitopes that are restricted to MHC-II, antigen-presenting molecules that are mainly expressed on professional APCs, including dendritic cells (DCs), macrophages and B cells , have been identified in various mouse tumor models as well as in cancer patients .

These MHC-II restricted neo-epitopes are presented to the TCR of CD4+ T cells, which upon activation can adopt various functions. Although a substantive CD4+ T cell response to a given neo-epitope is rather rare (~0.5% of neo-epitopes) , it is contended that neo-epitope-specific CD4+ T cells aid cancer regression in various ways, among others through direct killing of cancer cells and by supporting the priming, function and tumor infiltration of cancer-specific CD8+ T cells .

The observation that infusion of ex vivo expanded CD4+ T cells that recognized a neo-epitope derived from the mutated ERBB2IP, a gene encoding the ERBB2 interacting protein, into a patient with metastatic cholangio-sarcoma-induced significant tumor regression supports the importance of CD4+ T cells during the anti-cancer immune response . This notion is not new and has been considered for a long time during the development of cancer vaccines in which non-mutated tumor-associated antigens (TAAs) were used

4.2.2 Vaccine Resistance

Liu et al have noted:

Tumor intrinsic resistance

The resistance to cancer vaccines derives from intrinsic factors, including mutations in signaling pathways supporting tumor-immune control, downregulation or lost tumor antigen expression, altered antigen processing pathways, or loss of HLA expression. Above all could result in poor

recognition of tumor cells by T cells . The depletion of tumor antigens is a potential immune escape mechanism, especially for tumor cells whose survival could not be determined by the depleted antigens . The deletion of tumor antigens can be attributed to copying number loss at the genomic level or epigenetic changes. In addition, antigens loss also is mediated by immune selection.

Due to differences in immunogenicity among tumor cells, those tumors with strong immunogenicity induce an effective anti-tumor-immune response and will be eliminated by the body. Tumors with relatively weak immunogenicity can evade the immune system and selectively multiply, called immune selection. With continuous selection, the immunogenicity of the tumor becomes weaker and weaker. Additionally, low expression of HLA molecules and lacking presentation function are often lead to tumor-immune escape. HLA plays many roles in antigen processing and presentation.

Tumor cells can avoid T cell killing by downregulating the expression of HLA on the surface. Lack of costimulatory molecules is also a cause of tumor escape. The absence of costimulatory signals (such as B7, CD40, CD28) could cause the activation of T cells to fail and cause T cell tolerance. Furthermore, changes in tumor intrinsic signaling pathways may also cause immune escape . For instance, WNT/ β-catenin signaling pathway abnormal activation was associated with tumors that lack immune cell infiltration and are less likely to respond to immune checkpoint blockade .

Tumor extrinsic resistance

The extrinsic resistance of cancer vaccines may be caused by immunosuppressive cells in the immune microenvironment, including myeloid-derived suppressor cells (MDSCs) , tumor-associated macrophages (TAMs) , T regulatory cells (Tregs) , protumor N2 neutrophils, and cancer-associated fibroblasts (CAFs). Immunosuppressive cells interfere with the activation and proliferation of T cells by upregulating the expression of immunosuppressive receptors (such as PD1 or CTLA-4) and secreting immunosuppressive cytokines (such as IL-6, IL-10, TGFβ, and VEGF) [55, 56].

In addition, immunosuppressive cells can inhibit DC's function, promoting tumor resistance. MDSCs are pathologically activated neutrophils and monocytes with strong immunosuppressive activity . MDSCs are the cornerstone of the immunosuppressive barrier protecting tumors from the patient's immune system and immunotherapy . CAFs are the key component of the TME. CAFs can prevent the proliferation and migration of DC, recruit MDSCs, and inhibit T cell invasion by remodeling the extracellular matrix to construct dense fibrous stroma .

TAMs are classified as anti-tumorigenesis M1 (classically activated) and pro-tumorigenesis M2 (alternatively activated) phenotypes . TAMs were polarized into M2 macrophage by Th2 cytokines (IL-4, IL-10, TGFβ1) and immunocomplexes . M2 phenotype macrophages could promote the evolution of tumor related vasculature assist tumor cells in acquiring activation and remodeling stromal features to support tumors

4.2.3 Adjuvants

As Paston et al note:

Antigens alone in a vaccine are poor inducers of the adaptive immune response. In the absence of an adjuvant antigens targeted to immature DCs in the absence of inflammation or any microbial stimulation induce tolerance instead of a potent immune response . Adjuvants need to attract immune cells to the site of injection while also promoting cell mediated trafficking of antigen to draining lymph nodes and triggering the activation of APCs.

Current Vaccine Adjuvants The water-in-oil emulsions such as Montanide ISA 720 and Montanide ISA-51 have been widely adopted as adjuvants, they form a depot at the injection site, this results in the trapping of the soluble antigens preventing their rapid trafficking to local lymph nodes, this induces inflammation and the gradual release of the antigen. In a clinical trial Montanide ISA-51 was shown to induce both CD4+ and CD8+ T cell responses in patients vaccinated with long peptides of the oncoproteins E6 and E7 .

New vaccine adjuvants have been developed that target specific components of the immune system to generate a more robust and longer lasting immune response.

Newer adjuvants that consist of Pathogen-associated molecular pattern molecules (PAMPs) are now being used, these provide a danger signal that is recognized by pattern recognition receptors (PRRs) inducing an immune response. Innate cells express PPRs, these receptors include the Toll-like receptors (TLRs), nucleotide binding oligomerization domain like receptors and the mannose receptor. TLR agonists are increasingly being used as a vaccine adjuvant, they mimic microbial stimulation and have been shown to increase vaccine efficacy particularly for cancers .

Lymph node targeted TLR agonists have shown a direct relationship between the magnitude of CD8+ T cell responses and the amount of TLR agonist accumulated in draining lymph nodes, demonstrating the importance of providing sufficient inflammatory signals during immunization

4.2.4 Evasion

Van der Burg et al have noted evasion issues in cancer vaccines. They have summarized them as follows:

Tumour cell-intrinsic mechanisms: avoidance of immune recognition and elimination

• Defects in major histocompatibility complex (MHC) class I proteins (for example, genetic loss of the MHC locus)
• Epigenetic silencing of the antigen processing machinery (for example, transporter associated with antigen processing (TAP) and endoplasmic reticulum aminopeptidases (ERAPs))
• Loss of tumour-associated antigens (for example, dedifferentiation of melanoma cells)
• Shedding of alarm proteins (for example, ligands for the activating natural killer cell receptor D (NKG2D))

• Increasing resistance to cell death by immune cells (for example, increased expression of cellular FLICE-like inhibitory protein (cFLIP; also known as CFLAR) or inhibitor of apoptosis proteins (IAPs))
• Expression of ligands for inhibitory receptors (for example, programmed cell death protein 1 ligand 1 (PDL1), CD200 and human leukocyte antigen E (HLA-E))

Tumour cell-extrinsic mechanisms: creation of an immune-suppressive Microenvironment

• Infiltration with suppressive cells (for example, regulatory T cells, macrophages and myeloid-derived suppressor cells (MDSCs))
• Inactivation of immune receptors (for example, nitrylation of T cell receptor or binding of galectins)
• Deprivation of nutrients and oxygen (for example, indoleamine 2,3-dioxygenase (IDO) and effects of hypoxia)
• Secretion of immune-suppressive cytokines (for example, transforming growth factor-β (TGFβ), interleukin-10 (IL-10) and vascular endothelial growth factor (VEGF))

4.2.5 Which Antigen(s)?

As Lin et al have noted, there is a classification of various Ag. They discuss two major classes

Predefined antigens

Predefined antigens can be further classified by the frequency of expression across patient cohorts.

Shared antigens are those expressed in a sufficient proportion of patients such that vaccinologists can target these patient groups *(frequently within patient subsets of tumor types) using standard testing. Shared antigen vaccines can thus target both TSAs and TAAs. As examples, the neo-epitope TSA epidermal growth factor receptor variant III (EGFRvIII) is expressed in ~25% of EGFR-overexpressing glioblastomas (GBMs)19 and the viral TSA human papilloma virus E6 and E7 proteins (HPV E6 and E7) are expressed in ~60% of oropharyngeal cancers and nearly all cervical cancers, whereas the TAA Wilms' tumor protein (WT1) is overexpressed in most acute myeloid leukemias (AMLs), breast cancers and Wilms' tumors.*

Shared antigen vaccines are distinguished from personalized antigen vaccines in that the former can be assessed with standard testing such as cytology, immunohistochemistry and flow cytometry. Predefined, shared antigen vaccines have been the primary focus of preclinical and clinical research since the 1990s and have provided foundational lessons. Personalized antigens are unique to the vaccinated patient.

Personalized antigen vaccines have developed alongside the modern era of high-throughput gene sequencing and generally consist of TSA neo-epitopes *that, in contrast to the shared TSA EGFRvIII or Kirsten rat sarcoma virus (KRAS)G12D, are not sufficiently common to target a large group of patients. This approach allows the immune system to target tumors lacking known*

shared antigens but also places a burden on the vaccinologist to iteratively determine the optimally immunogenic epitopes.

Immunogenic epitopes must bind with sufficient avidity to both the peptide groove of an HLA molecule and to the complementarity-determining regions of a reactive T cell receptor (TCR)

Anonymous antigens ex vivo or in situ

*Instead of being classified by their antigen identity**, anonymous antigens can be classified by their method and location of APC loading.***

Anonymous antigen ex vivo vaccines are derived from excised tumor cells that are lysed and delivered to autologous APCs (Fig. 1b). Anonymous antigen in situ vaccines rely on endogenous APCs that are induced to uptake antigen at or near the tumor site, potentially following therapy-induced immunogenic cell death.

Contrary to predefined antigen vaccines, anonymous antigen vaccines may include a larger number of antigens and even new antigen types, such as peptide fusion epitopes86 and post-transcriptionally produced epitopes87, which are technically difficult to identify and not included in most neo-epitope pipelines.

Also Buonaguro and Tagliamonte have noted:

One of the principal goals of cancer immunotherapy is the development of efficient therapeutic cancer vaccines that are able to elicit an effector as well as memory T cell response specific to tumor antigens. In recent years, the attention has been focused on the personalization of cancer vaccines. However, the efficacy of therapeutic cancer vaccines is still disappointing despite the large number of vaccine strategies targeting different tumors that have been evaluated in recent years. While the preclinical data have frequently shown encouraging results, clinical trials have not provided satisfactory data to date.

The main reason for such failures is the complexity of identifying specific target tumor antigens that should be unique or overexpressed only by the tumor cells compared to normal cells. Most of the tumor antigens included in cancer vaccines are non-mutated overexpressed self-antigens, eliciting mainly T cells with low-affinity T cell receptors (TCR) unable to mediate an effective anti-tumor response.

In this review, the target tumor antigens employed in recent years in the development of therapeutic cancer vaccine strategies are described, along with potential new classes of tumor antigens such as the human endogenous retroviral elements (HERVs), unconventional antigens, and/or heteroclitic peptides. …

Targets for tumor vaccines fall into tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs). TAAs are self-antigens that are either preferentially or abnormally expressed in tumor cells, but may be expressed at some level in normal cells as well. T cells that bind with high affinity to TAAs are low in number because they are deleted by central and peripheral

tolerance mechanisms. Heteroclitic peptides are modified TAAs able to break tolerance and induce a more potent T cell response. TSAs include antigens encoded only by cancer cells and are truly tumor-specific, eliciting high-affinity T cells. Different colors indicate the difference between antigens presented …

Cancer immunotherapy has experienced tremendous progress in the last decade, including the dramatic expansion of our understanding of how cancer cells evade the immune system, and the development of several new therapies that are benefitting cancer patients. Therapeutic cancer vaccines offer an attractive alternative immunotherapy because of their potential safety, specificity, and long-lasting response due to stimulation of immune memory.

Unfortunately, many previous attempts to develop effective therapeutic cancer vaccines yielded disappointing results. Tumor antigens used so far all suffer from major drawbacks.

TAAs suffer from expression on normal cells and immunological tolerance, which can be overcome by designing appropriate heteroclitic epitopes.

TSAs represent the optimal target antigens but suffer from patient specificity, which hampers exploitation on a large scale.

Unconventional antigens may represent a great advancement, and their efficacy needs to be proven in clinical trials.

The accurate evaluation of the previous failures, combined with the constant technological improvements, will lead to the identification of the optimal tumor antigens. At the same time, the development of appropriate delivery strategies, adjuvants, and combination therapies to counteract the immunosuppressive tumor microenvironment will ultimately provide the sought improvement in the clinical outcome of Vaccines cancer patients.

4.3 ALTERNATIVES

There are other immune cells adapted to this approach.

4.3.1 NK Cells

In a recent paper Badrinath et al noted:

Most cancer vaccines target peptide antigens, necessitating personalization owing to the vast inter-individual diversity in major histocompatibility complex (MHC) molecules that present peptides to T cells. Furthermore, tumours frequently escape T cell-mediated immunity through mechanisms that interfere with peptide presentation. Here we report a cancer vaccine that induces a coordinated attack by diverse T cell and natural killer (NK) cell populations. The vaccine targets the MICA and MICB (MICA/B) stress proteins expressed by many human cancers as a result of DNA damage.

MICA/B serve as ligands for the activating NKG2D receptor on T cells and NK cells, but tumours evade immune recognition by proteolytic MICA/B cleavage. Vaccine-induced antibodies increase the density of MICA/B proteins on the surface of tumour cells by inhibiting proteolytic shedding, enhance presentation of tumour antigens by dendritic cells to T cells and augment the cytotoxic function of NK cells.

Notably, this vaccine maintains efficacy against MHC class I-deficient tumours resistant to cytotoxic T cells through the coordinated action of NK cells and CD4+ T cells. The vaccine is also efficacious in a clinically important setting: immunization following surgical removal of primary, highly metastatic tumours inhibits the later outgrowth of metastases. This vaccine design enables protective immunity even against tumours with common escape mutations.

4.3.2 Dendritic Cells

As Santos and Butterfield have noted:

Dendritic cells (DC) are specialized immune cells that play a critical role in promoting an immune response against Ags, which can include foreign pathogenic Ags and self-tumor Ags. DC are capable of boosting a memory T cell response but most importantly they are effective initiators of naive T cell responses. Many years of studies have focused on the use of DC vaccines against cancer to initiate and shape an anti-tumor specific immune response and/or boost existing spontaneous antitumor T cell responses.

In this study we give a brief overview of DC biology, function, and cellular subsets, and review the current status of the field of DC as cancer vaccines. …

DC are effective initiators of immune responses against self and non–self-antigens. In addition to phagocytosis and macropinocytosis, DC are equipped with a variety of receptors for Ag uptake.

Pathogens, tumor cells, and dying cells can be detected by DC through different molecules that serve as environmental sensors. After Ag uptake and processing, peptide Ags are presented to T cells via MHC I and MHC II complexes, whereas lipid Ags are presented through CD1 family molecules.

The expression of chemokine receptors allows DC to migrate to secondary lymphoid organs containing T cells. In addition to Ag presentation, DC also provide costimulatory signals for effective T cell activation.

Furthermore, DC can also produce cytokines that not only influence the type of T cell response generated, but also allow for cross-talk with other immune cells such as NK cells, macrophages, and B cells.

5 OBSERVATIONS

Reprogramming is a significant area of interest especially for cancer therapeutics. However it also presents a long list of issues that demand further investigation. We list and comment on some of these as follows.

5.1 WHAT ARE THE DYNAMICS OF MACROPHAGE REPROGRAMMING?

Macrophage reprogramming is an attempt to take starting cells which are malignant and convert them into macrophages to act as APCs. As such the intent is to have the cancer based macrophage produce surface Ag which the immune system can identify and then act upon. The details of this conversion and especially the production of Ag specific for the cancer are uncertain. One must also remember that the cartoons we draw to explain the process are limited in their ability to be dispositive. The cells surface is covered with receptors and proteins displaying the contents.

5.2 IS THERE A PROCESS WHICH INCORPORATE PLURIPOTENT REPROGRAMMING AND MACROPHAGE PROGRAMMING?

Pluripotent reprogramming is an attempt to reset the cell back to an embryonic state. Perhaps such a two step reprogramming may allow for a mor efficacious macrophage.

5.3 HOW DOES THE REPROGRAMMED CANCER CELL PRODUCE AND EXHIBIT THE ANTIGENS FOR UNIQUENESS?

Here the issue is one of internal to a cancer cell. Namely just how are the Ags in cancer cells produced? Are they parts of proteins that are unique to that cancer and more importantly to that specific cancer cell?

5.4 ARE THE ANTIGENS ELICITED FROM THE CANCER CELL SURFACE PROTEINS AS WELL?

A cancer cell may present a multiplicity of surface Ag but the immune system may fail to recognize them. As Abbas et al note:

Immune responses frequently fail to prevent the growth of tumors. There may be several reasons why antitumor immunity is unable to eradicate cancers. First, many tumors have developed specialized mechanisms for subverting host immune responses. We will return to these inhibitory mechanisms later in the chapter. Second, tumor cells lose the expression of antigens that may be recognized by the host immune system. Even tumors that do elicit effective immune responses may become less immunogenic over time because subclones that do not express immunogenic antigens have a selective survival advantage. Third, the rapid growth and spread of a tumor may overwhelm the capacity of the immune system to effectively control the tumor, which requires that all the malignant cells be eliminated. …

Tumor neoantigens are proteins encoded by mutated genes, which appear foreign to the immune system because they do not exist in normal cells and newly arise as a cancer develops. Usually, these neoantigens are encoded by genes carrying passenger mutations, which are point mutations or deletions that are unrelated to the development or malignant phenotype of the tumors.

The occurrence of passenger mutations reflects the genomic instability of cancer cells. Alternatively, a smaller number of neoantigens may be encoded by driver mutations in tumor-promoting oncogenes or tumor suppressor genes. Because T cells recognize only peptides bound to major histocompatibility complex (MHC) molecules, tumor neoantigens can be recognized only if peptides carrying the mutated amino acid sequences can bind to the patient's MHC alleles. Exome sequencing of many cancers has revealed large numbers of passenger mutations, and computer algorithms have been used to predict which of these mutations occur within peptide sequences that are likely to bind to the cancer patient's MHC alleles. Studies of cancer patients' T cells indicate that the tumor neoantigen peptides predicted to bind to MHC molecules in individual patients do, in fact, stimulate T cell responses in those patients, and that the number of different tumor antigen–specific T cell clones that are activated correlates with the number of mutations in the cancer.

5.5 WHAT IS THE VARIABILITY OF ANTIGENS BETWEEN INDIVIDUAL CANCER CELLS?

This is a significant issue. Not all cancer cells are the same. There may be a cancer stem cell, or just a mass of disparate cancer cells. This demands a cell by cell analysis of the malignancies. For example one may select Ags as targets that eliminate just cancer mass follower cells, aberrant but not controlling.

5.6 HOW DO MACROPHAGES ATTACK AND KILL CANCER CELLS?

Just what is the process by which macrophages identify and kill cancer cells produced by this reprogramming. We know that dendritic cells (DC) have been used and show some efficacy but why was a macrophage chosen. DC are effective APC and they activate the immune system in a better known manner.

5.7 IS THE REPROGRAMMING MOST EFFECTIVE ON HEMATOPOIETIC CELLS ONLY?

The results appear to favor hematopoietic cancers. If so, why? That is similar to CAR-T cell approaches.

5.8 IF THE DNA OF THE MALIGNANT CELL HAS BEEN CHANGED BY SOME MUTATION, DOES THIS REMAIN?

Changes in DNA in malignant cells such as translocation and hypermethylation remain in the reprogrammed cells. Does this not then crate a potential for secondary malignancies?

5.9 IF THE DNA OF THE MALIGNANT CELL CONTAINS THE REPROGRAMMED SEQUENCES SUCH AS PU.1, WHY NOT JUST ACTIVATE THAT?

The issue here is that the cancer DNA may already have the PU.1 gene. Why not activate the gene? If not, is suppressed and if so how?

5.10 WHAT OTHER MEANS ARE THERE TO EFFECTIVELY REPROGRAM?

In a recent paper noted by Phelan and Staudt the authors have noted:

Molecules have been developed that switch a transcription factor from being a repressor of gene expression to an activator — and thereby able to kill cancer cells. The findings offer a fresh strategy for designing anticancer drugs.

Cancer cells acquire genetic alterations that reprogram the expression of thousands of genes, to promote rapid cell growth and block pathways that induce cell death. … molecules that transform BCL6, a protein that promotes cancer by repressing the transcription of various genes, into a transcriptional activator. The molecules used to induce this transformation constitute a new class of compound for investigation as potential anticancer drugs. BCL6 functions as a master regulator of immune cells known as germinal centre B cells — which arise during normal immune responses, but are also the cells of origin for a type of cancer called diffuse large B cell lymphoma (DLBCL).

More specifically, BCL6 directly represses genes that encode cell-cycle inhibitors and proteins that are involved in a form of programmed cell death known as apoptosis. This protects germinal centre B cells and DLBCLs from stress caused by a process of programmed mutation (immunoglobulin somatic hypermutation) that allows the immune system to respond effectively to organisms that cause diseases.

Moreover, BCL6 maintains the identity of germinal centre B cells, preventing them from irreversibly differentiating into plasma cells by repressing the gene that encodes the transcription-repressing protein BLIMP-1. BCL6 is frequently dysregulated in DLBCLs, and thereby maintains the cancer cells in a highly proliferative state.

5.11 WHAT OTHER DISEASES ARE TARGETABLE

As Ye et al have noted:

Human iPS cells derived reprogrammed from patients with inherited neurodegenerative diseases, amyotrophic lateral sclerosis and Huntington's disease 79, have also been reported. Dimos et al., showed that they generated iPS cells from a patient with a familial form of amyotrophic lateral sclerosis. These patient-specific iPS cells possess the properties of ES cells and were reprogrammed successfully to differentiate into motor neurons. … iPS cells from fibroblasts of patient with Huntington's disease. They demonstrated that striatal neurons and

neuronal precursors derived from these iPS cells contained the same CAG repeat expansion as the mutation in the patient from whom the iPS cell line was established.

This suggests that neuronal progenitor cells derived from Huntington's disease cell model have endogenous CAG repeat expansion that is suitable for mechanistic studies and drug screenings. Disease specific somatic cells derived from patient specific human iPS cells will generate a wealth of information and data that can be used for genetically analyzing the disease. The genetic information from disease specific-iPS cells will allow early and more accurate prediction and diagnosis of disease and disease progression.

Further, disease specific iPS cells can be used for drug screening, which in turn correct the genetic defects of disease specific iPS cells.

iPS cells appear to have the greatest promise without ethical and immunologic concerns incurred by the use of human ES cells. They are pluripotent and have high replicative capability. Furthermore, human iPS cells have the potential to generate all tissues of the human body and provide researchers with patient and disease specific cells, which can recapitulate the disease in vitro. However, much remains to be done to use these cells for clinical therapy. A better understanding of epigenetic alterations and transcriptional activity associated with the induction of pluripotency and following differentiation is required for efficient generation of therapeutic cells. Long-term safety data must be obtained to use human iPS cell based cell therapy for treatment of disease

Also recently Grandos et al have focused this approach on melanomas.

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