



A Place for NK Cells in the Journey towards the Next Generation of Adoptive Immunotherapies

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Chimeric antigen receptors (CARs) have provided a powerful molecular mechanism to target T cells to specific surface antigens on tumor cells. The most promising results have been obtained by groups at the University of Pennsylvania, Memorial Sloan Kettering, and in a few additional small studies around the world, by achieving complete remissions in the 90% range in relapsed acute lymphoblastic leukemia (ALL) using T cells armed with CARs targeting the CD19 antigen. While the efficacy and durability of this cell therapy has been nothing short of astounding, we are now tasked to look beyond CD19 and apply this powerful approach to using cytotoxic cells to kill other types of tumors, including solid tumors which present many additional challenges for a CAR engineered cell.

In this brief overview, I would like to discuss several ways in which NK cells can offer us a path forward as a delivery platform for engineered cell (and ex vivo gene) therapy.

The purpose of this discussion is not to provide a detailed overview of NK cell biology. The apparent complexity of NK cell activation as well as the relative dearth of biomolecular insights as to the quantitative dynamics of NK cell activation also remains a challenge.

I would simply like to focus on four different aspects of cytotoxic cell therapy, and provide some contrasts where I feel NK cells can offer promise.

1. Durability

T cells have evolved to maintain their exquisite antigen specificity, as well as maximize the ability of the immune system to retain the benefits of the costly selection mechanism for the best clones by retaining a very small but critical subset as memory T cells to enable a potent recall response, even decades after the original expansion event. NK cells, for the most part, have eschewed such a purpose, as their primary role seems to be surveillance, early detection and essentially to hold the line until the reinforcements can arrive. As such, NK CAR therapies are unlikely to achieve the durability we have recently seen with CAR-T therapies for ALL. This apparent deficiency may actually prove to be an advantage for cell therapy. The flipside of durability can be toxicity (e.g., durable B cell depletion observed in some CD19 CAR-T studies), and periodic administration of NK CAR therapies according to a regular dosing regimen may provide a more accessible pharmaceutical model for the development of safer treatment options for a broader patient population.

2. Control

NK cell activation is negatively controlled by a diverse set of receptor families, such as KIR, NKG2A, and PD-1. In addition, tumor cells may downregulate ligands for these receptors, or upregulate ligands for activating NK receptors such as DNAM-1 and NKG2D, rendering certain tumors uniquely susceptible to NK cell lysis. Does this natural mode of NK cell-mediated tumor recognition potentially provide an additional mechanism to modify and control the specificity of NK cell therapies?

Do allogeneic NK CAR therapies offer a unique advantage? Since NK cells do not promote classic graft versus host disease (GVHD), a certain amount of "mismatch" between donor NK cells and host MHC class I and KIR receptor repertoires may provide enhanced efficacy for NK cell therapies. Matching of donor NK

haplotypes with patient genotype may offer a level of personalized cell therapy not accessible by CAR-T cell therapies. Guidelines established for ongoing clinical studies of adoptive transfer of related donor haploidentical allogeneic NK cells may be helpful in guiding future matching of donor and recipient haplotypes for engineered NK cell therapies.

3. Manufacturing

NK cells are few in number from primary sources of biological material such as cord blood, peripheral patient or donor blood and bone marrow. Their expansion conditions in culture are not well worked out, although combinations of certain cytokines (especially those binding to the gamma common chain receptor family) seem to offer promise of standardization without having to resort to irradiated feeder cells, and NK lines such as NK-92 offer a way to achieve industrial scale expansion. NK cells also seem to present additional challenges in cryopreservation and delivery, although these are early days and optimization efforts should offer improvements going forward. Design of the CAR itself is largely defined by domains involved in T cell activation, proliferation and survival, and using NK cells as the vehicle for CAR therapy may allow for a larger toolkit of domains to be incorporated into future iterations of CAR design. Bispecific linker molecules such as CD16/33 BiKE (bispecific killer cell engager) can also allow targeting of different tumor antigens. Finally, the cost of goods (COGs) for NK CAR therapies may be more amenable to commercial manufacturing, although, as of now, this is far from established.

4. Toxicity

CAR-T cell therapies have significant potential for on-target, off-tumor autoimmunity, and conditioning regimens (typically lymphocyte depletion with total body irradiation), while shown to promote CAR-T cell therapy efficacy across a broad range of clinical indications, can also present additional toxicity concerns. Could NK cell therapies be less reliant on lymphodepletion for reasons already mentioned, which will also reduce treatment burden and potential side effects of radiation therapy? Additional clinical data are needed to conclusively establish whether NK cell therapies will indeed be less dependent on "pre-conditioning." The anti-tumor response of CAR-T cell therapies is thought to be driven both by the cytotoxic potency and by cytokine-driven recruitment of additional endogenous immune elements. NK cell therapies will likely strike a different balance between these two elements. Although NK cells can be just as effective as T cells in killing tumor targets in vitro, a potential of NK cells to modify the immune environment in solid tumors may provide a unique advantage for CAR-armed NK cells in vivo through their unique interactions with dendritic cells or their ability to provide copious amounts of IFN gamma during the initial priming of immune response. The more ephemeral nature of NK cells also obviates the need to incorporate suicide elements into the CAR therapy approach. Overall, the lower toxicity anticipated by NK CAR therapies may give them an advantage in many therapeutic applications.

Through a focus on these four elements: durability, control, manufacturing and toxicity, we have sought to delineate some of the key aspects being discussed by the community today to enable broader use of NK cells in CAR-mediated engineered cell therapy technologies.



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