Chimeric Antigen Receptor Cell Therapy and Toxicity

There are an annual average of 69,740 new cases of non-Hodgkin lymphoma (NHL), 15,680 new cases of chronic lymphocytic leukemia (CLL) and 6070 new cases of acute lymphoblastic leukemia (ALL) in the United States, with estimated annual death rates of 19,020, 4580 and 1,430 respectively (http://www.cancer.org). Overall survival (OS) is determined largely by disease stage at presentation and response to chemotherapy or targeted therapy such as ibrutinib. Standard therapy for patients who relapse following frontline therapy is allogeneic hematopoietic stem cell transplantation (HSCT). The expected OS for patients in 2nd complete remission is 25% based on chemotherapy-sensitivity at the time of HSCT. Thus, there is an urgent and unmet need to develop new therapies for patients with advanced B-lineage malignancies, especially because relapse after transplant is usually fatal.

There is growing excitement over use of the body's own immune system (so-called cancer immunotherapy) to fight cancer. The past several years have seen tremendous advances in our understanding of the way through which the immune system guards against cancer and how cells can be engineered to enhance their activity. Adoptive cell therapy has emerged as a powerful treatment modality for advanced cancers refractory to conventional therapy. Chimeric antigen receptors (CARs) have been used extensively to redirect the specificity of autologous T-cells against lymphoid leukemia and lymphoma with striking positive clinical results.(1-6) B cell-derived malignancies are particularly well suited to targeting by CAR-expressing immune cells, since tumor cells almost invariably express CD19, an antigen physiologically expressed only by B lymphocytes.(7)

Although this therapy can induce rapid and durable clinical responses, it is also associated with unique acute toxicities, which can be severe or even fatal. The two most commonly observed toxicities with CAR T-cell therapies are cytokine release syndrome (CRS), characterized by high fever, hypotension, hypoxia and/or multiorgan toxicity, which may range in severity from low-grade constitutional symptoms to a high-grade syndrome associated with life-threatening multiorgan dysfunction; CAR-related encephalopathy syndrome (CRES) characterized by a toxic encephalopathic state with symptoms of confusion and delirium, and rarely, seizures and cerebral edema.(8-11) Although these toxicities are manageable in most patients, some require monitoring and treatment in the intensive care setting, and fatalities may occur. Such toxicities have also been observed after other redirected T-cell therapies such as TCR gene therapies or bispecific antibodies.

Patients at high risk for severe CRS include subjects with bulky disease, co-morbidities, and those who develop early onset CRS within three days of infusion.(12, 13) However, association of severe CRS with clinical parameters is imperfect and identification of biomarkers that predict severe toxicity are needed.

Genetic engineering of natural killer cells

Natural killer (NK) cells are another subset of lymphocytes that can be used for cancer immunotherapy.(14) They play a critical role in cancer immune surveillance and are the first line of defense against viruses and transformed tumor cells. NK cells have the intrinsic ability to infiltrate cancer tissue and their presence in the tumor is reported to correlate with better outcomes. Although most groups have relied on autologous or adult peripheral blood donor-derived NK cells for adoptive therapy, we have identified umbilical cord blood as an ideal source of NK cells because of their availability as a frozen product in cord blood banks worldwide, an advantage that has been bolstered by

methods to generate large numbers of highly functional NK cells from frozen CB units ex vivo.(15) This capability opens the way for an “off-the-shelf” source of NK cells that would improve the logistics of preparing and delivering NK cell-based treatments. It is however possible that CAR-engineered NK cells may also exert potentially serious toxicity, such as CRS or neurotoxicity, as reported with CAR T-cells. To counteract these potential complications, we have incorporated a suicide gene based on the inducible caspase-9 gene (*iC9*) into our CAR19 vector.(16) The addition of a small molecule dimerizer, AP1903, induces rapid apoptosis of transgenic cells both in vitro and in vivo, such that in the case of severe toxicity, the dimerizer could be introduced to induce apoptosis of CAR19-transduced CB-NK cells, allowing prompt resolution of the toxicity. This strategy would also be useful if the transduced NK cells are found to induce GVHD. Thus, we propose to perform the first-in-human clinical trial to test the safety and efficacy of CB-NK cells engineered to express a CAR against CD19, to ectopically produce IL-15 to support their in vivo proliferation and persistence, and express a suicide gene, based on *IC9*, that will address safety concerns related to the potential risk of direct toxicity

Criteria for Response to therapy

- Acute lymphoblastic leukemia: Complete response will be defined as bone marrow with < 5% blasts.
- CLL: Complete remission is defined as absence of clonal lymphocytes in the peripheral blood; absence of significant lymphadenopathy and no hepatosplenomegaly.
- Lymphomas:
  - Complete response: disappearance of all evidence of active tumor for a minimum of 8 weeks, without any symptoms.
  - Partial response: 50% or greater decrease in the sum of the products of measured lesions persisting for 4 weeks or more. No new lesions.
  - No change: responses inferior to "partial response", and/or no progression for a minimum of 8 week.
  - Progressive disease: appearance of new lesions or increase in the size of measurable disease. Subjects with progressive disease are not considered inevaluable for progressive disease.

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