Lymphodepletion: systemic chemotherapy with acute toxicities and detrimental long-term consequences for anti-tumor immune function.

The role of lymphodepletion in adoptive T-cell therapies

Current adoptive T-cell therapies, such as CAR T-cell therapies, are uniformly administered following a course of medications variously referred to as *lymphodepletion*, *lymphodepleting chemotherapy*, or *the lymphodepletion regimen*. Lymphodepletion is administered to a patient with the express intent of promoting the engraftment and expansion of the incoming adoptively transferred T-cells.

The concept of lymphodepleting chemotherapy was adapted from that used for autologous stem cell transplantation. Observations showed that adoptively transferred T-cells persisted longer and expanded more robustly in patients with very low levels of lymphocytes – a state termed "lymphopenia." Investigators working on adoptive cell therapy regimens thus attempted to replicate the lymphopenic state by administering drugs that were previously known to reduce lymphocyte counts. The resulting lymphodepletion regimens were designed to achieve substantial reductions in the absolute numbers and function of multiple types of host lymphocytes.

There are a number of mechanisms of action ascribed to lymphodepleting chemotherapy as it pertains to promoting the function of adoptive cellular therapy. Among those, one of the most important mechanisms is the reduction in competition for growth factors, enabling engraftment and expansion of the adoptively transferred cells. Lymphodepletion markedly reduces the number of host lymphocytes that take up trophic cytokines and growth factors that determine immune cell homeostasis. As a result, lymphodepletion frees up these cytokines to bind to and activate cytokine and growth factor receptors expressed on incoming adoptively-transferred therapeutic T-cells, leading to enhanced expansion and engraftment. In addition, lymphodepletion can reduce the rejection of the adoptively transferred cells by the host immune system, as there can be immune responses against autologous cells engineered to express transgenes that contain foreign sequences or anti-allograft responses in the context of donor-derived therapeutic cells.

However, while current lymphodepletion regimens are effective in promoting engraftment and expansion of adoptively transferred T-cells, the amount of cytokines and growth factors freed up is highly variable across patients, tumor types, and particular lymphodepletion regimens. This can contribute to variable cell therapy engraftment, with excessively rapid expansion of therapeutic cells resulting in toxicity events in some, while others experience suboptimal T-cell expansion and lack of efficacy. It is clear that while lymphodepleting chemotherapy is required for efficacy mediated in combination by the current class of adoptive cell therapies, it adds an

additional source of variability for safety and durable efficacy. The acute toxicities associated with lymphodepletion and/or the combined therapeutic cells requires close inpatient monitoring for the majority of patients. In addition, complex toxicity management protocols involving multiple targeted and non-targeted anti-inflammatory drugs are needed in severe cases. Promotion of adoptive T-cell expansion via lymphodepletion is thus a double-edged sword - increased expansion and durable efficacy are achieved, though often at the cost of clinical toxicity and logistical complications.

The cost of lymphodepletion

The extent of the toxic, systemic effects of lymphodepletion regimens is often overlooked. Most current regimens employ a combination of cyclophosphamide and fludarabine which, while commonly administered in the transplant setting, still cause significant morbidity and logistic administration issues. Cyclophosphamide acts by directly modifying DNA to inhibit DNA replication, while fludarabine acts as an anti-metabolite which is incorporated into DNA, resulting in stalling of DNA replication. When administered together, the combined mechanism of actions of these agents results in death of a substantial fraction of host lymphocytes, thus accounting for their frequent incorporation into lymphodepletion regimens. However, these mechanisms of action are associated with additional pleiotropic “on tissue” and “off tissue” effects that influence the short- and long-term clinical picture for patients.

While cyclophosphamide/fludarabine administration is effective in reducing host lymphocyte numbers and promoting engraftment of therapeutic T-cells, albeit with variable efficacy and associated toxicity, their on-tissue side effects are typically overlooked. Most notably, the elimination of some cancer-associated host lymphocytes is likely to be irreversible, often due to multiple coincident factors in addition to lymphodepletion. Regardless, lymphodepletion has permanent effects on host immune function because T-cells with unique recognition specificities that could target tumor cells or infectious diseases are permanently lost. T-cells with such specificities are not predictably replaceable, and depending on the age of the patient, may not be replaceable at all.

The loss of these specificities is highly relevant to patients with cancer, as multiple recent studies have demonstrated significant negative prognostic value of persistent lymphopenia in patients suffering from solid tumors. This provides a potentially important insight: systemic chemotherapy inhibits growth of solid tumors through direct targeting of tumor cells, while at the same time targeting proliferating cells of the immune system to detrimental effect. This latter effect of chemotherapy-induced immune damage manifests as lymphopenia and a reduction in the diversity of the repertoire of remaining T-cells. Collectively, the reduction in lymphocyte numbers and antigen recognition diversity compromise the capacity of the immune system to identify malignant cells. Thus, any form of systemic chemotherapy is a double-edged sword for people with cancer. It provides short term benefit by killing tumor cells and reducing the rate of tumor growth, while potentially compromising the potential of the patient’s immune system to maintain long-term tumor control by decreasing tumor surveillance

capabilities. It is also increasingly recognized that multiple courses of systemic chemotherapy have a cumulative effect on the immune system: the depth and duration of lymphopenia associated with administration of systemic chemotherapy increases with each course.

The implications of the negative prognostic significance of lymphopenia for use of lymphodepletion regimens in patients with solid tumors is clear. A course of lymphodepletion is an additional course of systemic chemotherapy that will compromise a patient's immune function, and use of repetitive courses of lymphodepletion will lead to cumulative immune damage. Because adoptively-transferred cells that are engrafted on top of lymphodepletion are typically narrowly targeting one or a few recognition specificities, the reduction in breadth of host T-cell populations results in a tradeoff: short-term benefit from the adoptively transferred T-cells vs. reduced potential for long term tumor control due to reduction of the endogenous anti-tumor immune repertoire should the tumor not be fully cleared.

Off tissue side effects of lymphodepletion may also be significant and comprise both short- and long-term toxicities. Lymphodepletion regimens are associated with varying degrees of myelosuppression with "off tissue" activity targeting hematopoietic progenitors resulting in reductions in neutrophil, platelet and red cell counts. With more intense lymphodepleting regimens being used to promote allogeneic T-cell engraftment, more prolonged myelosuppression is occurring in a larger fraction of patients. This results in concomitant increases in infection risk and the acuity of care of patients for one to two weeks until the LD-associated myelosuppression resolves. Myelosuppressive chemotherapeutic regimens are also associated with long term secondary malignancy risks related to induction of mutations in hematopoietic progenitors with long term self-renewal potential. Prolonged cytopenia and myelodysplasia are increasing areas of concern and research in cell therapy patients [1].

Looking to a lymphodepletion-free future

At Umoja, we believe that variable efficacy and toxicity in supporting cell engraftment, a long-term compromise of anti-tumor immunosurveillance activity of the host immune system, and substantial "off tissue toxicities" represent tradeoffs that do not have to be made when developing engraftment strategies for future cell therapies. Lymphodepletion has been linked to its capacity to free up cytokines such as IL-15 – as has been reported by multiple different groups [e.g. 2, 3, 4]. Relatedly, expression of cytokine receptors that provide an alternative source of growth and survival signaling has been shown to support cytokine independent engraftment of adoptively transferred T-cells [5]. Umoja's therapies have been developed in conjunction with use of our synthetic rapamycin-activated cytokine receptor (RACR) platform, which provides a non-lymphodepleting physician-controlled mechanism for supporting engineered T-cell populations (**Figure 1**).

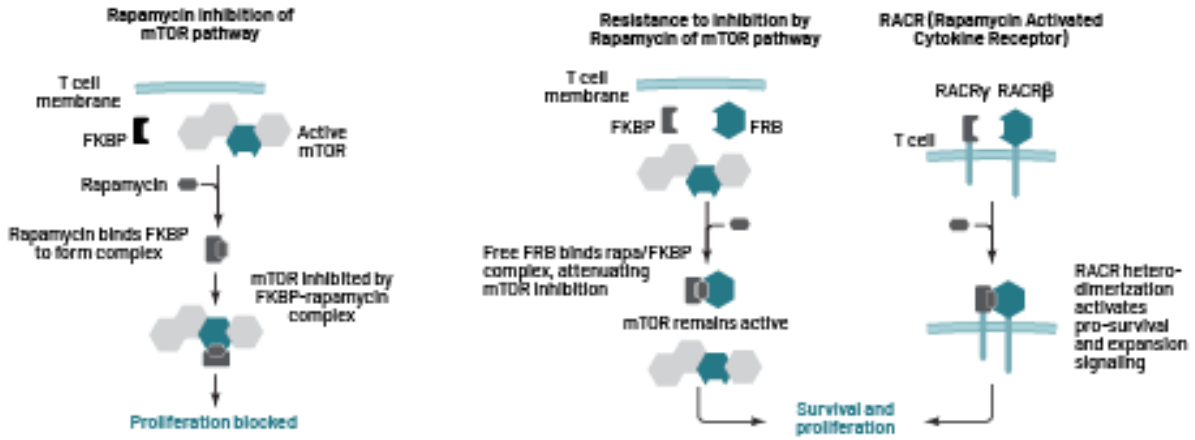

Figure 1: RACR (Rapamycin Activated Cytokine Receptor) signaling technology:

The rapamycin-activated cytokine receptor (RACR) system allows a patient's physician to give a well-tolerated FDA-approved drug (rapamycin) to activate growth and survival specifically in an engineered T-cell population, (right panels) while simultaneously inhibiting the proliferation of non-transduced T-cells (left panel).

The RACR system is designed to provide a uniform and consistent cell survival and expansion signal within and across patients. By making RACR signaling dependent on the presence of an exogenous drug we have incorporated what we expect will be an important safety mechanism, as discontinuing drug administration will cease RACR signaling, restoring normal immune cell function. Collectively, we believe that the RACR system represents a means of potentially safe, effective, controlled, and consistent support of the survival and expansion of engineered lymphocytes relative to non-engineered lymphocytes. In the near term, our goal is to deploy the RACR technology to limit the dependence of adoptive T-cell therapies on lymphodepletion and its attendant side effects. Our long-term vision is the development of adoptive cell therapies which are able to durably engraft and generate significant clinical impact independently of any form of lymphodepleting chemotherapy.

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