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Prospects for immunotherapy of acute myeloid leukemia using $\gamma\delta$ T cells

“Perhaps the time is now right to evaluate $\gamma\delta$ T-cell immunotherapy in patients with acute myeloid leukemia, particularly in that large majority of patients who are unsuitable for allogeneic hematopoietic stem cell transplantation.”

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The majority of T lymphocytes express an $\alpha\beta$ T-cell receptor (TCR) that detects processed peptide antigen, presented in the context of MHC antigens. However, a significant minority population (generally <10%) express an alternative TCR, comprising γ and δ subunits. The distinctive nature of these cells has been appreciated since their discovery and initial characterization, in the early to mid-1980s. Activation of $\gamma\delta$ T cells is commonly triggered by engagement of diverse molecular markers of cellular stress or transformation. Consistent with this, the inter-relationship between these elusive T cells and cancer has preoccupied translational investigators for several years. Early studies demonstrated that $\gamma\delta$ T cells undergo oligoclonal expansion in patients with malignant disease, can infiltrate neoplastic lesions and provide immune surveillance against tumor formation in mice [1]. Remarkably, the presence of intra-tumoral $\gamma\delta$ T cells has recently been ranked as the single most prognostically favorable immune-cell infiltrate identified across a panel of 39 tumor types [2]. Furthermore, the MHC unrestricted nature of antigen recognition by these cells means that they do not cause graft versus host disease, theoretically facilitating their safe ‘off-the-shelf’ or allogeneic use. Balancing this however, $\gamma\delta$ T cells sometimes act as willing conspirators in the malignant process – an event that may be accompanied by IL-17 release and/or deployment of one or more immunosuppressive functions, such as

galectin-1 release [3,4]. These considerations highlight the challenge imposed to effectively harness these cells for immunotherapeutic purposes.

$\gamma\delta$ T cells may be viewed as the ultimate multi-taskers or ‘jack of all trades’ of the immune system. They straddle innate and adaptive immunity, and display features that are reminiscent of professional antigen-presenting cells, natural killer (NK) cells, conventional cytotoxic $\alpha\beta$ T cells and/or suppressive regulatory T cells. Once activated, $\gamma\delta$ T cells deploy several effector mechanisms to destroy target cells, including the release of perforin and granzymes, inflammatory cytokines (e.g., IFN- γ and TNF- α), TRAIL and Fas ligand. These cells also provide an integrated and anatomically compartmentalized surveillance system that operates within the bloodstream and the tissues. In man, these two roles are broadly served by subsets that respectively express a $\gamma\delta$ TCR containing a V δ 2 or a non-V δ 2 subunit (most commonly either V δ 1, V δ 3 or V δ 5). However, these distinctions do not appear to be absolute. For example, although V δ 1⁺ T cells are generally the dominant $\gamma\delta$ tumor-infiltrating population in melanomas, it is the proportion of infiltrating V δ 2⁺ (but not V δ 1⁺) T cells that correlates with earlier disease stage and reduced risk of metastatic spread [5]. Conversely, enhanced reconstitution of V δ 1-enriched $\gamma\delta$ T cells following allogeneic hematopoietic stem cell transplantation (aHSCT)

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is associated with improved survival in patients with acute myeloid leukemia (AML) [6]. Underscoring this complexity, it has recently been proposed that diverse butyrophilin-like molecules regulate the maturation and expansion of locally operative $\gamma\delta$ T-cell subtypes in distinct epithelial tissues [7]. For these reasons, both V δ 2⁺ and non-V δ 2⁺ subsets warrant consideration as therapeutic effectors against malignant disease. The focus of this editorial is to assess prospects for $\gamma\delta$ T-cell immunotherapy of AML – a disorder that continues to increase worldwide and which accounted for approximately 265,461 deaths in 2012 [8].

In the majority of AML patients, disease remission can be achieved using combination chemotherapy. Curative consolidation is then achieved most effectively using a cellular immunotherapy, namely aHSCT. Unfortunately, this option is unavailable to most patients, either due to lack of a suitable donor or comorbidity and risk of transplantation-related mortality. It is logical therefore that investigators have sought to develop alternative cellular immunotherapies that combine this level of therapeutic potency with improved safety and universality of application. Against this background, it is opportune to ask how well $\gamma\delta$ T cells might measure up to this task?

The best studied $\gamma\delta$ subset is that which is most accessible, namely the V γ 9V δ 2 (also termed V γ 2V δ 2) T cells that predominate in the bloodstream. These cells recognize phosphoantigen (PAG) intermediates of the mevalonate pathway, notably isopentenyl pyrophosphate. Given that aberrant mevalonate metabolism contributes directly to transformation [9], it is not surprising that increased flux through this pathway leading to enhanced PAG production is commonly found in AML blasts [10]. Accumulation of PAG is further potentiated by aminobisphosphonate agents, such as zoledronic acid, accounting for its propensity to cause transient expansion of these cells and febrile reactions/flu-like symptoms in many patients [11]. Recognition of PAG is dependent upon butyrophilin-3A1, although additional intermediates appear to be required to fully reconstruct this antigen-presenting machinery.

The ability of V γ 9V δ 2 T cells to detect leukemic and other abnormal cell types is strengthened by their expression of a series of complementary innate receptors, many of which are shared with NK cells. Pre-eminent among these is NKG2D, a C-type lectin that binds the stress ligands, MICA/B and ULBP family members. V γ 9V δ 2 T cells also express DNAM-1, which interacts with nectin-2 (CD112) and polio virus receptor (nectin-like 5; CD155).

Evidence that V γ 9V δ 2 T cells exert meaningful control over AML is supported by a number of clinical observations. The combination of zoledronic acid

and low dose IL-2 was administered to patients with a range of refractory malignancies, with the goal of activating the endogenous V γ 9V δ 2 T-cell population. Notably, the only individuals that benefited from this intervention had refractory/relapsed AML (two partial responses and two stable disease in seven evaluable patients) [12]. Small scale studies in which haploidentical $\gamma\delta$ T cells and zoledronic acid have been infused in patients with AML and other hematologic malignancies have also resulted in disease remission [13].

Preclinical evidence also provides support for this contention. Leukemic blasts express ligands for NKG2D and DNAM-1 and can be killed *in vitro* by V γ 9V δ 2 T cells [14,15]. Furthermore, V γ 9V δ 2 T cells traffic to bone marrow and have been shown to retard progression of an aggressive AML xenograft [14]. Blocking studies using antibodies and mevalonate pathway inhibitors indicate that TCR and DNAM-1 engagement are the most important interactions in triggering the killing of AML cells [14,15]. By contrast, NKG2D ligands, such as MICA/B and ULBP family members are generally expressed at lower levels on leukemic blasts. Nonetheless, ULBP-1 expression predicts sensitivity of AML cells to killing by allogeneic V γ 9V δ 2 T cells, whereas nectin-2 expression correlated negatively in this regard [16].

Non-V δ 2 $\gamma\delta$ T cells are primarily resident within epithelial tissues where they protect against infection (including viral reactivation) and transformation [4]. Many are responsive to stress ligands, cytomegalovirus (CMV) and other viruses, while some cross-react with poorly characterized tumor-associated molecules that include endothelial protein C receptor. Other non-V δ 2 cells engage glycolipid antigens that are presented on tumor and other cell types by CD1 family members, notably CD1c and CD1d [5]. Adding to complexity, hybrid receptors have also been described in which V δ 1 segments are fused with segments from the TCR α chain family.

CMV-responsive V δ 1 cells are commonly increased in patients following aHSCT, owing to CMV reactivation. Intriguingly, this is associated with reduced relapse of disease, suggesting that these cells also exert intrinsic antileukemic activity [15]. In keeping with this, V δ 1 $\gamma\delta$ T cells also demonstrate *in vitro* cytotoxic activity against AML cells and this is further enhanced following CMV reactivation [15,17]. While molecular interactions remain to be fully characterized, suspicion falls on the TCR itself in addition to the broad array of innate receptors expressed by these cells, including NKG2D, DNAM-1 and NK receptors that include Nkp30, Nkp44 and Nkp46 [18].

Pathways forward can be envisioned for the clinical evaluation of both V δ 1 and V δ 2 subsets in the

experimental immunotherapy of patients with AML. V γ 9V δ 2 T cells are traditionally considered to be easier to expand *in vitro*, although methods have recently been described to allow efficient, convenient and clinically compatible expansion of V δ 1⁺ T cells from peripheral blood [19]. Furthermore, V δ 1 cells are reported to be more resistant to activation-induced cell death than their V δ 2 counterparts [20]. Balancing this however, V γ 9V δ 2 T cells have a natural propensity to traffic to sites of disease in AML. Furthermore, immunotherapy using these cells can be potentiated by aminobisphosphonates, such as zoledronic acid. These agents sensitize target cells to attack by V γ 9V δ 2 T cells and are naturally tropic for bone, creating a drug reservoir close to the site of disease origin. Further enhancement of aminobisphosphonate delivery to sites of malignant disease can be achieved using liposome-encapsulated formulations, which exploit the abnormally leaky neovasculature that is found in many solid tumors and hematological malignancies [21]. Providing additional therapeutic options, $\alpha\beta$ T cells can be genetically engineered to express $\gamma\delta$ TCRs of either the V δ 1 or V δ 2 subtype, thereby conferring their antileukemic specificity upon these cells [17]. To date, most clinical trials of $\gamma\delta$ T-cell immunotherapy have focused on solid tumors, where efficacy has been limited. This contrasts with their better-studied first

cousins, NK cells, which have been extensively studied for their antileukemic activity. Perhaps the time is now right to evaluate $\gamma\delta$ T-cell immunotherapy in patients with AML, particularly in that large majority of patients who are unsuitable for aHSCT.

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