The cure rate and 10-year event-free survival (EFS) for multiple myeloma remains low despite the introduction of high-dose chemotherapy supported by autologous peripheral blood stem cell transplantation (auto-PBSCT) and the application of novel drugs. New therapeutic modalities that are non-cross-resistant with chemotherapy, such as immunotherapy with natural killer (NK) cells, are clearly needed to improve outcome, particularly in patients with chemotherapy-resistant myeloma.1,2 We have shown that chemo-resistant myeloma cells can be killed by killer-cell immunoglobulin-like receptor (KIR)–ligand mismatched (KIR–L MM) NK cells from an allogeneic, haplo-identical donor. In addition, we have transfused haplo-identical KIR–L MM NK cells in the setting of an auto-PBSCT in a pilot trial of 8 patients with advanced myeloma. This immunotherapy proved to be safe and did not cause graft versus host disease (GvHD) or rejection of the autograft. However, although these initial clinical results are encouraging and suggest that complete responses can be induced, the NK cell doses transfused are likely not sufficient to destroy a large myeloma burden.

We now propose building on these initial results with a 3-pronged approach for enhancing the clinical efficacy of NK cell therapy for myeloma (Figure 1). We hypothesize that we can further improve the killing of chemo-refractory myeloma with KIR–L MM NK cells by activating and expanding the NK cells and by modulating the interaction between NK effectors and myeloma targets. To test this hypothesis, we will pursue the following specific aims.

**Specific Aim 1:** Determine if NK cell dose and potency can be reliably increased by expanding and activating NK cells prior to infusion. Expansion of NK cells is important if we are to overcome the myeloma burden. Without expansion, there will be too few NK cells and too many myeloma cells to eradicate all chemo-refractory myeloma. Prior to infusion, we will stimulate KIR–L MM haplo-identical NK cells with K562 cells transfected with membrane-bound IL15 and the co-stimulatory molecule 4-1BB ligand. We contend that NK cells can be expanded >100-fold and be highly activated to kill primary myeloma with this in vitro manipulation.

**Specific Aim 2:** Evaluate whether antibody-dependent cellular cytotoxicity (ADCC) of NK cells can be enhanced by flagging myeloma cells for NK cell detection and elimination. We will use a humanized antibody to CS-1, a CD2 receptor family molecule expressed by myeloma cells but not by normal tissues, to confer myeloma-specific NK cell cytotoxicity. This antibody will effectively ‘flag’ myeloma cells for ADCC-mediated killing by NK cells. It has been well established that antibodies, such as rituximab (anti-CD20), have potent anti-lymphoma effects through ADCC.4 We hypothesize that anti-CS1 will have a similar effect in myeloma.

**Specific Aim 3:** Determine if myeloma vulnerability to NK cell killing can be increased by downregulation of inhibitory ligands on myeloma cells. Over 50% of NK cells from KIR–L MM donors are non-alloreactive to myeloma cells due to the presence of human leukocyte antigen (HLA)–class I ligands on myeloma cells, which interact with inhibitory receptors on NK cells, preventing myeloma cell kill. However, NK cells avidly kill targets that either do not express or only weakly express HLA-class I. A prime example is the NK-sensitive cell line K562, which lacks expression of HLA-class I. We propose that by treating patients with proteasome inhibitors, we should be able to downregulate HLA-class I on myeloma cells, which will then allow killing of myeloma by all NK cells, including non-alloreactive NK cells. This will greatly increase the number of NK cells available to destroy the myeloma burden.

Successful implementation of our 3-pronged strategy for enhancing the efficacy of NK cell therapy for myeloma should make myeloma cells as susceptible to killing as the highly NK-sensitive cell line K562. Results of these studies can then be used to improve our current protocol employing KIR–L MM NK cell therapy for patients with refractory myeloma. In future, this treatment approach could be applied in the autologous setting as additional frontline therapy for newly diagnosed myeloma patients. In addition, this research could lead to more efficacious treatment for other NK cell–sensitive malignancies.