Biological Characterization and Differential Gene Expression Analysis of CYT-338 NK Cell Engager (NKE) Against CD38 Expressing Tumors Including Multiple Myeloma (MM)

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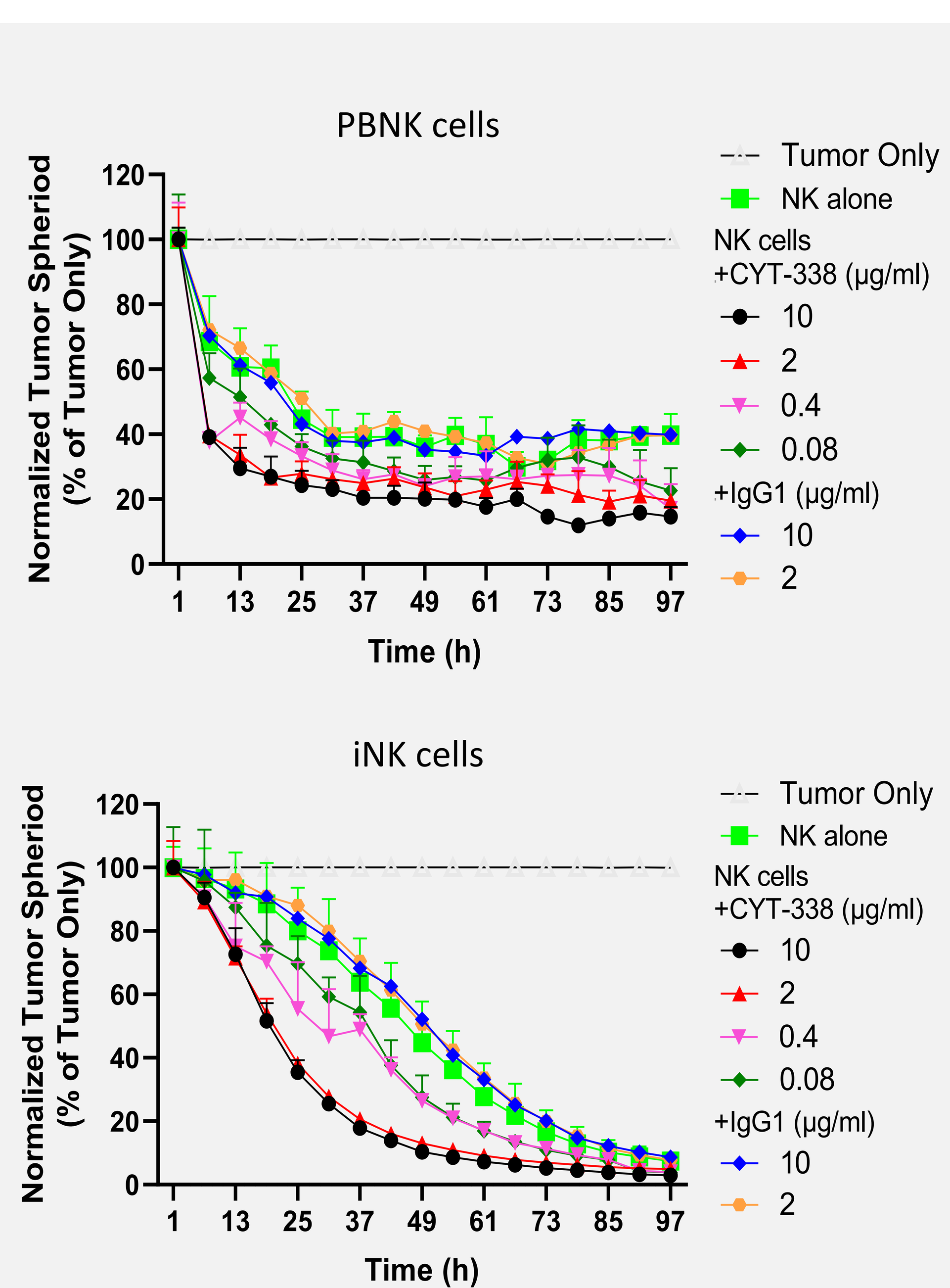
INTRODUCTION

CYT-338 is a cell-engager bispecific antibody designed using Cytovia's proprietary FLEX-NK™ platform to redirect NKp46 activating receptor expressing NK cells to kill CD38 expressing Multiple Myeloma (MM) tumors. The human IgG1 backbone provides additional Fc effector functionality to mediate antibody dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP) and complement dependent cytotoxicity (CDC) against CD38 positive tumors. Here, we further characterized CYT-338 tumor cytotoxicity and showed potent cytolysis of MM tumor spheroids in the presence of Cytovia's iPSC derived NK (iNK) or peripheral blood NK cells (PBNKs). We also showed how CYT-338 enhanced iNK serial killing of MM tumors and reversed NK cell dysfunction and exhaustion. Finally, we compared anti-tumor effects of CYT-338 and daratumumab in primary MM patient samples ex-vivo via multi-omic profiling and subsequent differential gene expression analysis to elucidate potential mechanisms contributing to the increased NK cell cytotoxicity observed with CYT-338.

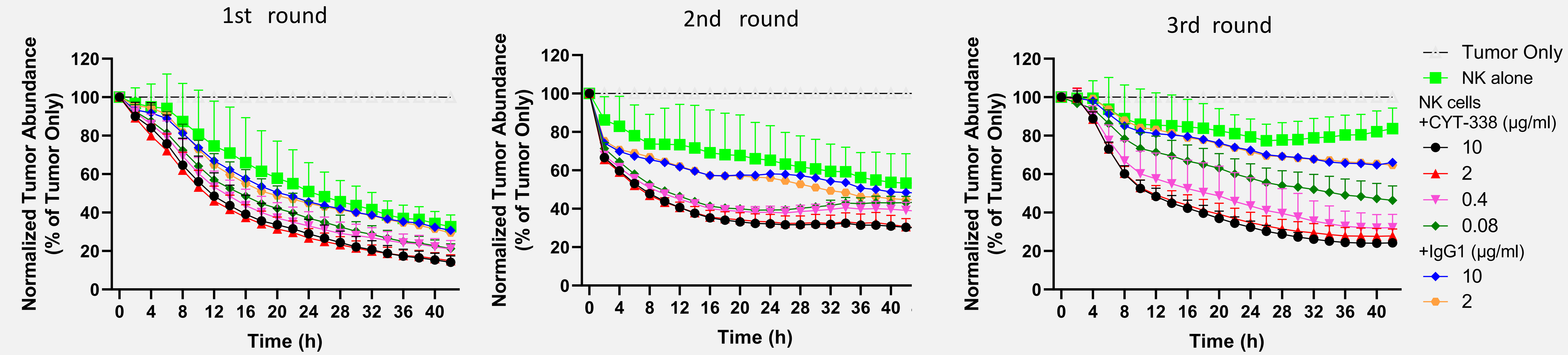
METHODS

MM.1S-GFP tumor spheroids were established in ultra-low attachment U-bottom plates and killing assays were conducted with iNKs or PBNKs using the Incucyte ™ Live Cell Analysis System. MM1S tumor cell serial killing assays were conducted for 3 rounds of 42h by repeatedly adding the same iNK or PBNKs alone or in combination with CYT-338 to fresh tumor cells following each round of tumor killing. CYT-338 Fc effector function against MM1S tumors was evaluated for ADCP using human macrophages differentiated from purified monocytes isolated from peripheral blood and for CDC in the presence of rabbit complement. The cytotoxicity of CYT-338 and daratumumab was evaluated using newly diagnosed MM patient CD38+ tumor cells and autologous NK cells purified from bone marrow and peripheral blood and co-cultured in a customized microfluidic platform for 24 hrs. Differential gene expression using mRNA isolated from these cultures was measured using Nanostring's nCounter® using the Pan cancer IO360™ gene panel. Gene expression pathway analysis was conducted using Principal component analysis.

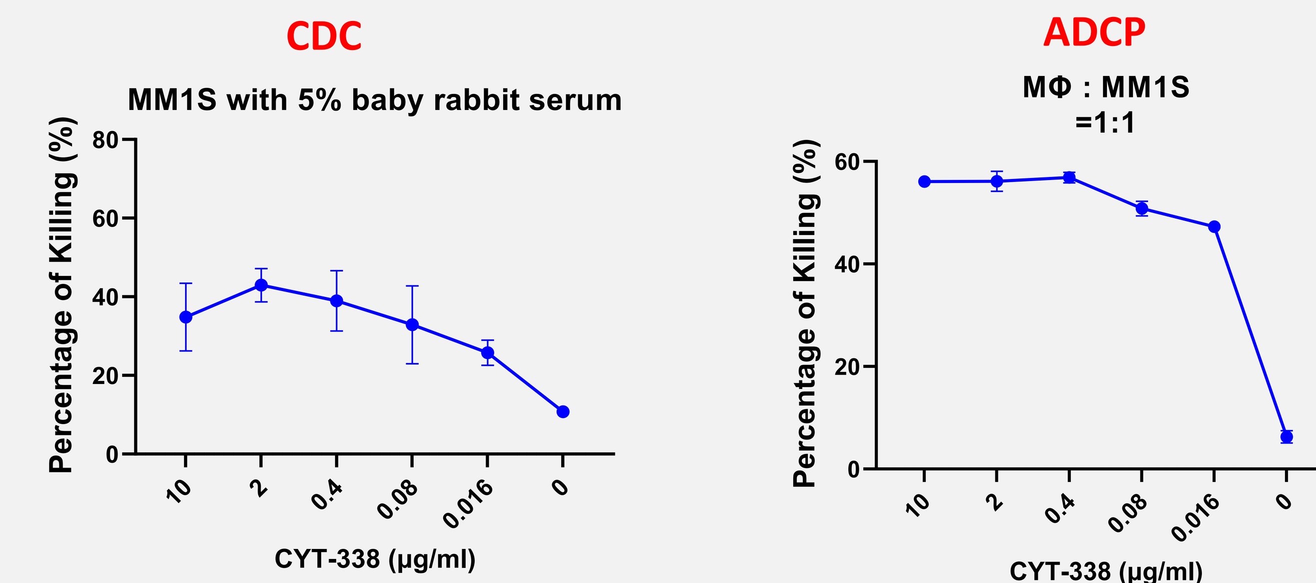
RESULTS



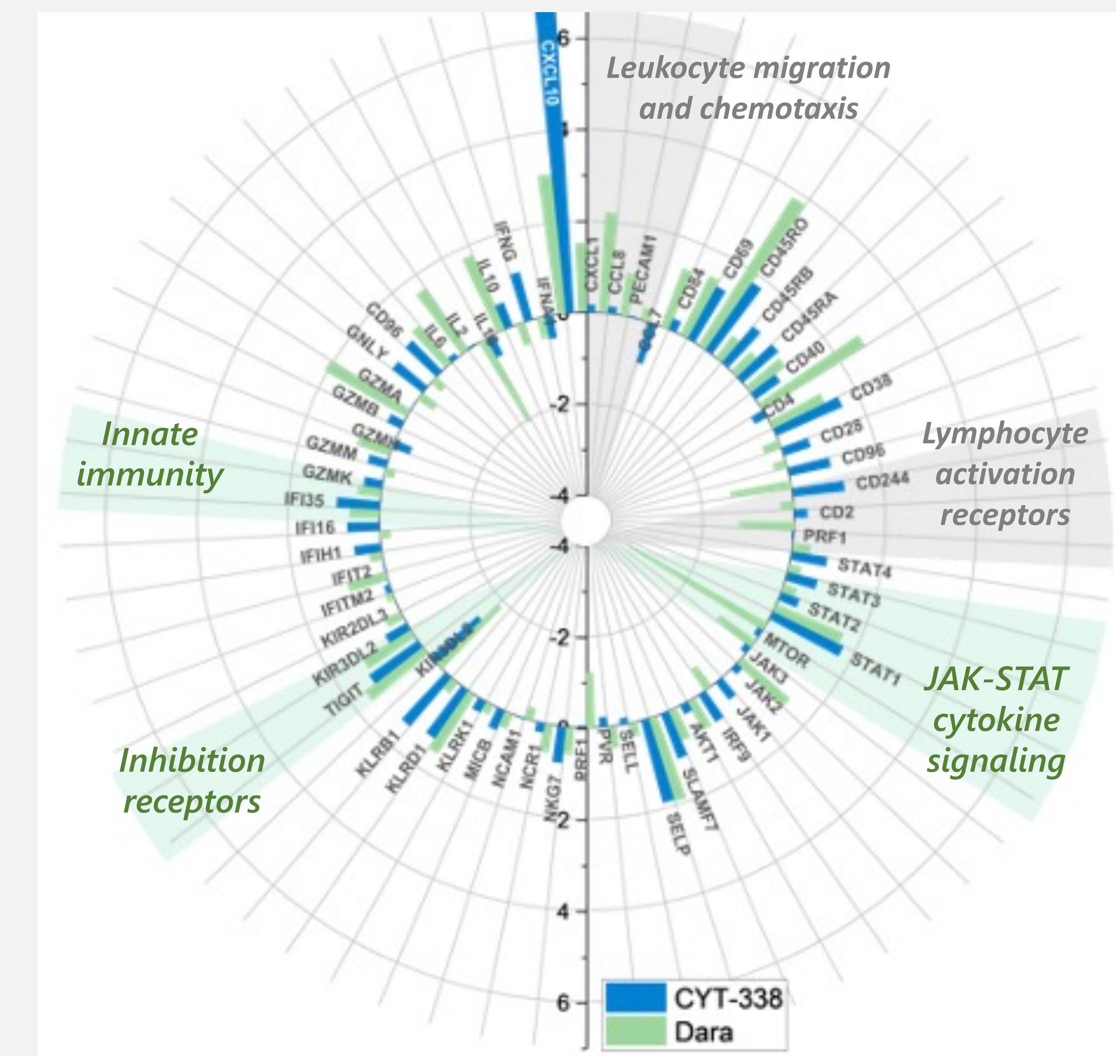
1. CYT-338 enhances iNK and PBNK cytolysis of multiple myeloma tumor spheroids.
MM.1S-GFP tumors were cultured for 2 days in ultra low attachment U-bottom plates to form tumor spheroids. NK cells and different concentrations of CYT-338 were added to the tumor spheroids and tumor killing was assessed over time by evaluating reduction of GFP positive tumor spheroids using the Incucyte™ system.



2. CYT-338 enhances iNK serial killing of multiple myeloma tumors and reverses NK dysfunction.
Serial killing of iNK against MM1S-GFP tumors was evaluated with either iNK alone or in combination with CYT-338 using fixed E/T=1:2 ratios at each round of killing. Tumor lysis was monitored by assessing reduction of GFP positive tumors using the Incucyte™ system.



3. CYT-338 induces macrophage mediated antibody dependent cellular phagocytosis and complement dependent cytotoxicity of MM tumors.
MM1S-GFP tumors were incubated with CYT-338 and baby rabbit serum containing complement for 4 hrs and tumor lysis assessed by flow cytometry using cell viability dye (on the left). Macrophages were differentiated from purified monocytes following culture with M-CSF for 5 days. CYT-338 induced macrophage phagocytosis of MM1S-GFP tumors was assessed in a 4 hr assay using viability dye staining of tumors by flow cytometry (on the right).



4. CYT-338 and Daratumumab induced similar and distinct gene expression profiles in patient NK and MM cell co-cultures. Gene ontology enrichment analysis following treatment with CYT-338 and daratumumab showed increased expression of innate immunity and cytokine signaling pathway related genes (eg. IFI35, GZMK, JAK/STAT). CYT-338 treatment showed highest enrichment for lymphocyte and leukocyte activation receptor related genes (eg. CD96, CD244, and CD2), while daratumumab showed highest enrichment for leukocyte migration and chemotaxis genes (eg. PECAM-1, CCL8, CXCL1).

CONCLUSIONS

- CYT-338 increased the ability of iNK and PBNKs to kill MM tumor spheroids in a dose dependent manner.
- Serial killing activity of iNKs against MM tumors declined over additional rounds of killing but the combination with CYT-338 maintained serial killing at high levels suggesting the ability of CYT-338 to overcome NK cell exhaustion and dysfunction.
- CYT-338 showed potent dose dependent ADCP against MM tumors indicating an additional effector cell pathway targeted by CYT-338.
- CYT-338 showed dose dependent CDC against MM tumors demonstrating the complement activation pathway targeted by CYT-338.
- Gene expression analysis of autologous patient NK cell and MM co-cultures treated with CYT-338 or daratumumab showed similar and distinct gene expression profiles. The distinct lymphocyte activation gene expression pathways activated by CYT-338 may contribute to the increased NK cell redirected cytotoxicity of MM tumors compared to daratumumab.
- The above results support further development of CYT-338 as a potent NK cell engager that could also activate macrophages by using ADCP mechanism and complement to mediate its anti MM tumor effects.