Tumoricidal Effects of Macrophage-Activating Immunotherapy in a Murine Model of Relapsed/Refractory Multiple Myeloma

Jeffrey Lee Jensen1,2, Alexander Rakhmilevich2,3, Erika Heninger1,2, Aimee Teo Broman4, Chelsea Hope1,2, Funita Phan2,5, Shigeki Miyamoto2,5, Ioanna Maroulakou6, Natalie Callander1,2, Peiman Hematti2,5, Marta Chesi7, P. Leif Bergsagel7, Paul Sondel2,3,8, and Fotis Asimakopoulos1,2

Abstract

Myeloma remains a virtually incurable malignancy. The inevitable evolution of multidrug-resistant clones and widespread clonal heterogeneity limit the potential of traditional and novel therapies to eliminate minimal residual disease (MRD), a reliable harbinger of relapse. Here, we show potent anti-myeloma activity of macrophage-activating immunotherapy (αCD40+ CpG) that resulted in prolongation of progression-free survival (PFS) and overall survival (OS) in an immunocompetent, preclinically validated, transplant-based model of multidrug-resistant, relapsed/refractory myeloma (t-VeMYC). αCD40+CpG was effective in vivo in the absence of cytolytic natural killer, T, or B cells and resulted in expansion of M1-polarized (cytolytic/tumoricidal) macrophages in the bone marrow. Moreover, we show that concurrent loss/inhibition of Tpl2 kinase (Cot, Map3k8), a MAP3K that is recruited to activated CD40 complex and regulates macrophage activation/cytokine production, potentiated direct, ex vivo anti-myeloma tumoricidal activity of αCD40+CpG-activated macrophages, promoted production of antitumor cytokine IL12 in vitro and in vivo, and synergized with αCD40+CpG to further prolong PFS and OS in vivo. Our results support the combination of αCD40-based macrophage activation and TPL2 inhibition for myeloma immunotherapy. We propose that αCD40-mediated activation of innate antitumor immunity may be a promising approach to control/eradicate MRD following cytoreduction with traditional or novel anti-myeloma therapies.

Cancer Immunol Res. 3(8); 881–90. ©2015 AACR.

Introduction

Despite the advent of novel therapies for multiple myeloma, a cancer of mature lymphocytes that produce antibody, the disease remains incurable. Only one in three patients diagnosed with myeloma will be alive 10 years after the diagnosis (1). Lack of curative approaches is attributable to early and near-universal clonal heterogeneity (2, 3), and the persistence of minimal residual disease (MRD) following traditional and novel therapies, including high-dose therapy with autologous stem cell rescue. Recent evidence has confirmed that detectable MRD constitutes a harbinger for relapse and predicts adverse clinical outcomes (4, 5). Thus, eradication of MRD is a priority in designing curative approaches against myeloma.

We have proposed that therapeutic manipulation of the microenvironment may be an essential component of curative interventions in myeloma (6). Proof-of-principle for this concept is provided by the fact that the only known potentially curative therapy for myeloma, allogeneic transplantation, exerts its effects through modulation of the microenvironment to produce a graft-versus-myeloma effect, albeit at the cost of considerable toxicity (7). Therefore, strategies to render the microenvironment inhospitable or overtly hostile to myeloma cells are urgently needed.

Macrophages are a crucial, and somewhat neglected, component of the myeloma niche. We have previously shown that myeloma-associated monocytes/macrophages (MAM) are key modulators of the inflammatory milieu of the myeloma niche and important producers of cytokines that are known to promote growth of nascent myeloma tumors (8). MAM continue to produce inflammatory cytokines (IL1β, IL6, and TNFα) even as they acquire tumor-promoting ("M2") characteristics. This "mixed" or "intermediate" state of macrophage polarization in vivo is similar to previously described "M2b macrophages" or "MSC-educated macrophages" (9–11).

We and others have shown that therapeutic macrophage repolarization (toward an M1-tumoricidal phenotype) using CD40 ligation can be harnessed to exert antitumor activity in vivo (12–21). Therapeutic activation of macrophages typically requires two sequential signals, a "priming signal" delivered...
through agonistic CD40 stimulation and a secondary “triggering signal” delivered through Toll-like receptor (TLR) stimulation. The resultant tumoricidal activity has been shown to be independent of T cells and has shown promise even in very difficult cancers: Thus, clinical administration of αCD40 agonist monoclonal antibody, without TLR activation, has demonstrated clinical benefit in patients with pancreatic cancer, acting through macrophage activation (21). Macrophages are particularly attractive as anti-myeloma effectors because of their association with myeloma lesions outside the bone marrow (22) where active MRD may localize.

We have demonstrated a role for control of macrophage polarization and cytokine production by TPL2, a MAP3K operating at the interface between NFκB and MAPK pathways (8, 23). Moreover, we have attributed roles for Tpl2 in myeloma progression in vivo and have shown that Tpl2 activity promotes macrophage polarization toward pro-tumor (M2) phenotype. Tpl2 is activated by stimuli that activate macrophages (such as TLR ligands and CD40; ref. 24), but its actions limit the production of crucial antitumor effectors (such as nitric oxide; ref. 25) or antitumor immunomodulatory cytokines [such as IL12 (ref. 26) and IFNγ (ref. 27)]. At the same time Tpl2 promotes promyeloma immunomodulatory cytokines IL1β, IL6, and IL10 (8). Tpl2 signaling could, therefore, be envisaged as an “innate immune checkpoint” that modulates innate anti-myeloma immunity.

In this article, we show that αCD40-mediated macrophage repolarization results in potent anti-myeloma activity both ex vivo and in vivo. Host Tpl2 loss promoted production of IL12, an M1-polarization agent and a powerful antitumor cytokine (28) and prolonged survival. Our results may open new avenues for controlling myeloma relapse by harnessing the power of innate antitumor immunity.

### Materials and Methods

#### Antibodies and reagents

The FGK45.5 hybridoma–producing αCD40 was a gift from Dr. F. Melchers (Basel Institute for Immunology, Switzerland). The endotoxin content of our FGK45.5 preparation was directly quantified using the E-Toxate Kit (Sigma) and was found to be below detection limit [0.05–0.1 endotoxin units (EU) per mL]. We have previously reported that FGK45.5–activated macrophages harvested from endotoxin-resistant C3H/HeJ mice retained potent ex vivo antitumor activity, suggesting that macrophage activation was not the result of inadvertent endotoxin contamination of αCD40 antibody (20). Endotoxin-free CpG1826 (TCCATGACGTTCCTGACGTT) was purchased from Coley Pharmaceuticals Group. Monophosphoryl Lipid A (MPL), L-NG-nitroarginine methyl ester (L-NAME; final concentration, 5 mM/L), and rat IgG were purchased from Sigma. Pan-caspase inhibitor carbobenzoxy-Val-L-Val-Asp-fluoromethylketone (Z-VAD-FMK; final concentration, 20 μmol/L) was purchased from Promega.

#### Ex vivo macrophage cytotoxicity assays

Mice received 0.5 mg αCD40 FGK45.5 or rat IgG (Sigma, H4131) i.p. and 3 days later peritoneal exudate cells (PEC) were obtained by peritoneal elution. Red blood cell lysis was performed by rapid exposure to deionized water. PECs were seeded onto 96-well plates and allowed to adhere for 90 minutes. MM1.S-mCherry/Luc cells and 5 μg/mL of CpG or MPL were subsequently added to adherent PECs. MM1.S-mCherry/Luc cells were a generous gift from Dr. Constantine Mitsiades (Dana-Farber Cancer Institute, Boston, MA). Tumor cell viability in PEC/tumor co-cultures was assessed after 48 hours by adding sterile-filtered luciferin (Promega; P1043) diluted in RPMI complete media to a final concentration of 250 μg/mL, incubating the co-culture at 37°C 5% CO2 for 30 minutes, and then measuring luminescence using a BioTek Synergy4 plate reader. Supernatant nitrite levels were measured using a Griess Reagent-based colorimetric assay (Sigma, G4410). Cytokine levels were measured using the bead-based BioPlex System (Bio-Rad). Transwell experiments were performed using inserts with 3.0-μm diameter pores, a polycarbonate construction, and a separation distance of 0.127 cm between the bottom of the insert and the top of the plate (Corning: 3385).

#### In vivo tumor model

Vk12598 tumor cells were described previously (29). Tumor cells were injected via intracardiac injection at a dose of 3 × 105 CD138+ cells per recipient mouse, without irradiation (day 0). Mice were treated with intraperitoneal, saline-dissolved injections of 0.25 mg αCD40 or rat IgG on day 5, and 25 μg CpG or PBS on day 8 post-injection, repeated every 2 weeks. Tumor burden was assessed using serum obtained after clotting 20 μL of blood obtained from tail grasps. Sera were analyzed for monoclonal gammapathy using a Sebia Hydrasys/Gelscan system. M-spike band/total protein ratio was multiplied by the total protein content of the serum, as determined using Bradford reagent, yielding absolute quantification of monoclonal gammapathy. Mice were bled and analyzed weekly starting at day 17. Mice that never showed any gammapathy (potential lack of engraftment) were excluded from analyses. Treatment with αCD40+CpG did not affect the engraftment rate (~85%).

#### Statistical analysis

All in vitro experiments were performed in triplicate and statistical significance between groups in a single experiment was assessed using the two-tailed Student t test. ANOVA methods were used to test differences between groups across multiple independent experiments. For in vivo experiments, progression-free survival (PFS) was defined by a monoclonal gammapathy <0.5 g/dL. Time to progression was determined by linearly interpolating between the two measured gammapathy time points above and below 0.5 g/dL. Kaplan–Meier survival curves were analyzed using the log-rank test and using a Cox proportional hazards model for estimating the interaction between αCD40+CpG and Tpl2 status.

### Table 1. Log-rank test comparing survival curves in Fig. 4

<table>
<thead>
<tr>
<th>Kaplan–Meier curve comparisons</th>
<th>OS</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>αCD40−/− vs. αCD40+/+</td>
<td>P = 0.432</td>
<td>P = 0.548</td>
</tr>
<tr>
<td>αCD40−/− vs. αCD40+/+</td>
<td>P = 7.12 × 10−6</td>
<td>P = 7.81 × 10−7</td>
</tr>
<tr>
<td>αCD40−/− vs. αCD40+/+</td>
<td>P = 0.0169</td>
<td>P = 0.00870</td>
</tr>
</tbody>
</table>

Abbreviations: OS, overall survival; PFS, progression-free survival.
Results

Macrophages can be activated to exert tumoricidal activity against myeloma cells \textit{ex vivo}

To confirm that macrophages possess intrinsic anti-myeloma activity, we collected PECs 3 days after \textit{in vivo} priming with αCD40 or control rat IgG antibody. We confirmed that adherent PECs represent peritoneal-origin macrophages: CD115 expression was used to confirm their monocytic identity—more than 95% of adherent PECs expressed CD115 (data not shown). PECs were seeded in 96-well plates to which luminescent myeloma MM1.S cells (MM1.S-mCherry/Luc) were subsequently added at various ratios of PECs to target cells (effector:target, E:T ratio) (30). TLR agonists or PBS were added to the \textit{in vitro} co-cultures, as described in Materials and Methods. Emitted bioluminescence exhibited an almost perfectly linear correlation to the absolute number of myeloma tumor cells in each well when myeloma cells were cultured in isolation or in co-cultures with nonluminescent PECs ($r^2 = 0.99$, Fig. 1A).

Addition of TLR agonists CpG (TLR9) or MPL (TLR4) to myeloma cell mono-cultures did not result in appreciable toxicity (Fig. 1B). By contrast, tumoricidal effects of activated PECs were observed in PEC–myeloma cell co-cultures. TLR stimulation alone, in the absence of prior αCD40 stimulation, was only effective at higher E:T ratios, particularly with the potent TLR4 agonist MPL (Fig. 1B). Prior stimulation of PECs with αCD40 significantly enhanced the tumoricidal activity of PECs, at lower E:T ratios, in the presence of either CpG or MPL. The most potent antitumor cytotoxicity was obtained by αCD40-primed macrophages that were stimulated with MPL. However, CpG stimulation also produced significant antitumor cytotoxic effects in a dose-dependent manner (Fig. 1B). Myeloma cell demise was associated with apoptosis induction. Macrophage-mediated cytotoxicity was abrogated in the presence of the pan-caspase inhibitor Z-VA-D-FMK (Supplementary Fig. S1A) and Annexin V/propidium iodide (PI) staining corroborated apoptotic mechanisms (Supplementary Fig. S1B).

The observed cytotoxic effects of different combinations of stimuli correlated with the degree of nitric oxide (NO) production by activated macrophages. Thus, the most potent tumoricidal combination (αCD40 and MPL at 20:1 E:T ratio) also resulted in the most enhanced NO production (Fig. 1B). To determine whether NO was necessary for the observed antitumor activities by PECs, we blocked NO production with L-NAME, a pan-NO synthase inhibitor. Treatment with L-NAME resulted in attenuation of NO production in conjunction with attenuation of antitumor cytotoxic effects (Fig. 1C). We conclude that activated macrophages can exert cytotoxic effects against myeloma cells \textit{ex vivo} that are partially NO dependent.

Prior reports have suggested a role for cell-to-cell contact in mediating protective effects of macrophages toward myeloma cells (31). Moreover, direct phagocytosis of myeloma cells by
macrophages has also been shown (32). We tested the hypothesis that cell–cell contact or juxtacrine interactions may be necessary for the observed antitumor effects of activated macrophages. To test this hypothesis we repeated the experiments shown in Fig. 1B with the addition of a Transwell separator that prevents contact between PECs and myeloma cells. As shown in Fig. 1D, separation of PECs from myeloma cells attenuated the antitumor effect.

These results demonstrate that activated macrophages exert anti-myeloma activity through direct cell–cell contact or alternatively/additionally, through juxtacrine interactions.

Tpl2 loss enhances tumoricidal activity of activated macrophages ex vivo

BecauseTpl2 loss promotes spontaneous macrophage repolarization in the myeloma microenvironment in vivo (8), we hypothesized thatTpl2 loss/inhibition might synergize with therapeutic macrophage repolarization to enhance myeloma cell killing by activated macrophages. Initially, we tested this hypothesis in the ex vivo cytotoxicity assay described above. We compared the relative cytotoxic activity ofTpl2+/− PECs withTpl2−/− PECs. As shown in Fig. 2A,Tpl2−/− macrophages exhibited enhanced cytotoxicity compared withTpl2+/− macrophages at each condition tested. Both TLR4 stimulation (MPL) and TLR9 stimulation (CpG) enhanced cytotoxicity byTpl2−/− PECs relative to cytotoxicity byTpl2+/− PECs.

Tpl2 activity has been shown to repress transcription of inducible NO synthase (iNOS) following TLR stimulation in vitro (25). We found thatTpl2 loss also enhanced NO production byεCD40+TLR-stimulated PECs (Fig. 2B). Moreover, NO levels directly correlated with enhanced cytotoxicity exhibited byεCD40+TLR ligand-treated PECs (Fig. 2A and B).

We next tested the hypothesis thatTpl2 loss might enhance production of antitumor cytokine IL12 byεCD40+TLR-stimulated macrophages. IL12 and IL10 have been considered markers ofM1 and M2 macrophage polarization, respectively.Tpl2 signaling has been shown to exert repressive effects on IL12 transcription following TLR9 stimulation in vitro (26), but the impact of this observation on antitumor immunity has been unclear. We found thatTLR9 (CpG) but not TLR4 (MPL) stimulation led to increased IL12p40 production byεCD40-activated macrophages.
that exert anti-myeloma activity ex vivo (Fig. 2C). Whereas Tp2 loss enhanced IL12p40 in both macrophage mono-cultures and macrophage–myeloma cell co-cultures, no IL12p40 production was found in myeloma cell mono-cultures. IL10 production was not affected by Tp2 genotype (Fig. 2C).

Tumoricidal effects of αCD40+ CpG-activated macrophages require intact NFκb1 activity

Tp2 associates with NFκb1 (p105), a core component of the NFκB canonical signaling pathway (33). p105 is the precursor of the active NFκB subunit p50. Tp2 kinase activity is repressed when Tp2 is bound to p105. Signals that activate the canonical NFκB pathway lead to p105 degradation, and thus to Tp2 release and activation. Because Tp2 is only stable in association with NFκb1, we hypothesized that therapeutic macrophage activation may exert antitumor effects in "minimal disease" states in myeloma. To model the growth and progression of MRD in an immunocompetent host, we used a transplant-based model in which a threshold inoculum of tumor cells was delivered on June 17, 2017. © 2015 American Association for Cancer Research. cancerimmunolres.aacrjournals.org Downloaded from cancerimmunolres.aacrjournals.org on June 17, 2017. © 2015 American Association for Cancer Research.
myeloma growth. Injection of Vk12598 cells through the intracardiac route into immunocompetent syngeneic C57BL/6j recipients, without preconditioning irradiation, results in universal death in engrafted animals (median, 32 days, 95% confidence interval, 30–37) from progressive myeloma (Fig. 4A). The rapid clinical course allowed overall survival (OS) to be determined as a study endpoint. Moreover, the multidrug-resistant profile of Vk12598 (29) offers an ideal vehicle to test the concept that therapeutic macrophage activation can inhibit drug-resistant, proliferative myeloma.

Treatment of animals with αCD40 + CpG resulted in significant prolongation of OS that was further enhanced inTpl2-null recipients. Figure 4B shows a significant positive effect of treatment on prolongation of OS (progression defined as M-spike >0.5 g/dL), which was further enhanced by recipientTpl2 loss. Log-rank statistical significance values are given in Table 1. Cox proportional hazard analysis (as detailed in the Supplementary Data) estimated that, among αCD40 + CpG-treated animals, wild-type genotype conferred a relative risk of 2.21 for death (P = 0.02) and 2.22 for progression (P = 0.02), compared with that of theTpl2-null genotype (Supplementary Tables S1 and S2).

We hypothesized that the improvement in OS and death conferred by recipientTpl2 loss may be related to enhanced IL12 production in vivo. To test this hypothesis, we measured serum levels of IL12p40 at 4 hours and 24 hours following administration of αCD40 + CpG in vivo. We found that administration of αCD40 + CpG led to a modest increase in IL12p40 inTpl2−/− animals. By contrast,Tpl2 loss led to sustained production of significantly higher amounts of IL12p40 that persisted 24 hours after treatment (Fig. 4C). IL10 levels did not differ significantly betweenTpl2−/− andTpl2−/− treated animals (Fig. 4D). Consistent with our hypothesis, treatment with αCD40 + CpG did not significantly affect levels of pro-myeloma inflammatory cytokines IL18 and IL6 (Supplementary Fig. S2), demonstrating that macrophage-activating immunotherapy did not lead to a surge of pro-myeloma inflammatory cytokines. Moreover, Vk12598 myeloma cells express low levels of CD40, a feature typical of human advanced myeloma (34; Supplementary Fig. S3).
In vivo activity of αCD40+CpG is independent of cytolytic NK or T-cell activity

Although we have shown that macrophages can exert anti-myeloma activity in vivo following αCD40+TLR stimulation, we asked whether αCD40+CpG acts primarily through macrophages in vivo. To this end, we transplanted Vk12598 cells into SCID/Beige animals, characterized by defective NK-cell cytolytic activity and absence of mature T or B cells, the latter due to the SCID mutation (35). Vk12598 cells were injected on day 0 and treatment was administered as per the schedule delineated in Materials and Methods. Tumor burden was assessed by serum protein levels of monoclonal gammapathy (myeloma tumor burden) between αCD40+CpG-treated SCID/Beige and C57BL/6J immunocompetent recipients.

The results are shown in Fig. 5. αCD40+CpG treatment was at least partially effective in the absence of cytolytic NK, T, or B cells (Fig. 5), strongly supporting a central role for macrophages in mediating the in vivo anti-myeloma effects of αCD40-based immunotherapy.

We have previously demonstrated that macrophages in de novo Vk’MYC-tumor-bearing animals partition into a CD68hi/Ly6Chigh population that expresses iNOS and is consistent with M1-like macrophages and a CD68hi/Ly6Chigh population that does not express iNOS and is consistent with M2-like macrophages (8). A similar immunophenotyping strategy was used for analysis of myeloid cell subpopulations in the present study with some modifications: First, we eliminated F4/80 staining for the initial gating on the basis of a recent report favoring Ly6C and Ly6G in lieu of F4/80 stain for analysis of mouse splenic myeloid cell populations (36). Second, we introduced IL4Rx (CD124) to better characterize the M2-like subpopulation (37).

Treatment with αCD40+CpG led to expansion of the CD68hi/Ly6Chigh population expressing iNOS (M1-like) (Fig. 6). Figure 6 also shows the data from analysis of all animals in the untreated and αCD40+CpG-treated cohorts in two independent experiments. These results demonstrate that αCD40+CpG leads to expansion of the M1-like macrophage population in myeloma-infiltrated bone marrow in vivo.

Discussion

The incidence of multiple myeloma continues to rise, with an estimated 24,050 new cases in 2014 in the United States (1). Its precursor form, monoclonal gammapathy of undetermined significance (MGUS), is the most common hematologic disorder with a prevalence of 4% in the general population over the age of 40 years. Despite the advent of novel therapies that have revolutionized the treatment landscape, most patients diagnosed with myeloma will die of their disease. Novel therapies and stem-cell transplantation confer excellent cytoreduction and may prolong survival. Whereas current clinical research aims to define the role of autologous transplant in the era of novel therapies, it is clear that none of these approaches are routinely curative (38).

The lack of curative approaches in this disease reflects the persistence of residual myeloma acting as a nidus for regrowth and clinical relapse. In recent years, quantification and characterization of this MRD have received more attention (39). It is now generally accepted that the detection of measurable MRD portends an ominous prognosis (4, 5).

Although there is growing appreciation for the correlation between MRD and clinical outcome, biologic characterization is
still incomplete. Characterization of MRD is likely to be complicated by significant inter- and intrapatient heterogeneity. We have envisaged distinct types of MRD that may be further complicated by overlapping mechanisms: First, MRD may reflect the persistence of cells that have acquired genetic or epigenetic attributes of drug resistance through a process of classical, linear clonal evolution. Second, MRD may represent 'tides' involving subclones that have arisen through a process of 'branching' evolution (40). Third, MRD may reflect the specific biologic attributes of clonogenic precursors of the disease. Although the nature of these "myeloma stem cells" is hotly debated, they are likely to be relatively resistant to the effects of therapy (41, 42). Alternatively, MRD may reflect the stochastic persistence of residual myeloma cells with tumor-protective niches.

Activation of selected components of the tumor's microenvironment to maximize efficacy and minimize toxicity may be an essential component of approaches aiming at eradication of MRD and achievement of cures. Strategies to mobilize the microenvironment against residual myeloma should work with little regard to the clonal composition of MRD. Among various components of innate immunity in the myeloma niche, our work has focused on monocytes/macrophages (6). Whereas the concepts governing polarization and plasticity of macrophages in solid tumors have been well formulated (43, 44), the mechanisms governing macrophage behavior in hematologic malignancies are not as well understood. Multiple myeloma, a malignancy in which tumor cells are critically dependent on cross-talk with diverse components of the bone marrow microenvironment, provides an excellent model to study interactions between macrophages and tumor cells and evaluate the potential of their therapeutic exploitation.

We have previously shown that monocytes/macrophages provide essential support to tumor cells in the nascent myeloma lesion through the elaboration of critical promyeloma cytokines such as IL1β and IL6 (8). We have proposed that approaches to limit production of these cytokines may be useful in controlling indolent myeloma. The Weissman laboratory has previously shown that macrophages may exist in a state of precarious equilibrium with myeloma cells. Simple blockade on protective "don't-eat-me" signals on the surface of the myeloma tumor cells suffices to elicit potent anti-myeloma, macrophage-mediated cytotoxicity (32). Whereas blocking "don't-eat-me" signals constitutes a "passive" approach in turning macrophages into anti-myeloma effectors, "active" methods to repolarize macrophages may hold even better therapeutic promise. We have previously demonstrated that active repolarization of macrophages to elicit tumoricidal activity can be achieved through the administration of sequential CD40-mediated activation and TLR ligation (12, 19). CD40-induced macrophage activation has been shown to result in meaningful clinical tumor regressions in recalcitrant solid tumors (e.g., pancreatic cancer; ref. 21) and in a model of chronic lymphocytic leukemia, a tumor of mature B lymphocytes (15).

In this study, we demonstrate that macrophages can be induced to elicit potent anti-myeloma tumoricidal activity both ex vivo and in vivo. Activation of murine macrophages through administration of CD40 with sequential TLR activation in vitro led to dose-dependent cytotoxic activity against myeloma cells. Gene loss of the MAP3K, Tpl2, promoted the tumoricidal activity of activated macrophages. The increased cytotoxic potential of Tpl2-null macrophages correlated with higher production of tumoricidal effectors, such as NO. Importantly,
TPL2 inhibition offers an attractive adjunct to therapeutic macropage-mediated tumor cell killing, but may have also led to amplification of anti-myeloma response through additional effects on NK and T-cell–mediated antitumor immunity.

Therapeutic macrophage activation led to prolongation of disease PFS and OS in a model of relapsed/refractory myeloma, t-VkMYC (29, 45). We have demonstrated that the high proliferative rate, aggressive clinical course, and drug resistance of t-VkMYC accurately model end-stage, relapsed/refractory myeloma (29). The use of a drug-resistant t-VkMYC model allowed us to test the hypothesis that therapeutic macrophage activation may be effective in controlling drug-resistant myeloma. This model may have important clinical implications given the paucity of clinical approaches to treat drug-resistant, end-stage myeloma.

Weakly agonistic or antagonistic cCD40 antibodies have shown modest clinical activity and an acceptable safety profile in multiple myeloma (46, 47). In this article, we provide a rationale for strongly agonistic cCD40 immunotherapy in myeloma. TPL2 inhibition could provide an ideal adjunct to strongly agonistic cCD40 immunotherapy when the tumor cells express CD40. CD40-mediated MAPK pathway activation is dependent upon TPL2 activity: B cells from Tpl2−/− mice fail to activate ERK in response to CD40 stimulation (48). Therefore, TPL2 inhibition may prevent a cell-autonomous growth-promoting effect of cCD40 on CD40-expressing myeloma cells, while also further activating CD40-bearing macrophages to elicit tumoricidal activity.

In summary, we propose that therapeutic macrophage repolarization coupled with TPL2 inhibition may be a promising approach to control drug-resistant residual disease in myeloma. TPL2 inhibitors in advanced pharmaceutical development (49). We have previously proposed single-agent TPL2 inhibition as monotherapy to delay progression of indolent myeloma through interference with elaboration of pro-myeloma cytokines by MAM (8) and potentially other microenvironmental cell types. Advanced myeloma may be less responsive to TPL2 monotherapy because of acquisition of autocrine cytokine support and/or mutations in crucial signaling pathways, for example, NF-kB mutations, considered to be progression events (50). Therapeutic activation of macrophages may be an effective means to control progression of relapsed/refractory myeloma relapse. Moreover, TPL2 inhibition offers an attractive adjunct to therapeutic macrophage activation in multiple myeloma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


www.aacrjournals.org Cancer Immunol Res; 3(8) August 2015 889

Authors’ Contributions

Conception and design: J.L. Jensen, I. Maroulakou, P. Sondel, F. Asimakopoulos
Development of methodology: J.L. Jensen, E. Heninger, P. Hematti, M. Chesi, F. Asimakopoulos
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.L. Jensen, E. Heninger, C. Hope, I. Maroulakou, M. Chesi, P.L. Bergsagel, F. Asimakopoulos
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.L. Jensen, A. Rakhmilevich, E. Heninger, A.T. Broman, F. Asimakopoulos
Writing, review, and/or revision of the manuscript: J.L. Jensen, A. Rakhmilevich, A.T. Broman, C. Hope, F. Phan, S. Miyamoto, I. Maroulakou, N. Callander, P. Hematti, P.L. Bergsagel, P. Sondel, F. Asimakopoulos
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Phan, P. Hematti
Study supervision: F. Asimakopoulos
Other (wrote the first draft of the article): J.L. Jensen
Other (design of experiments): A. Rakhmilevich
Other (provided support, feedback, and exchange of ideas through weekly group meetings): F. Phan

Acknowledgments

The authors thank the Emery Bresnick and Lixin Rui laboratories for help with experiments and equipment. The authors thank Tyler Van De Voort and Xueyi Qu for help with the experiments. The authors thank Dr. Constantine Mitsiades (Dana-Farber Cancer Institute) for providing MM1.S-mCherry/Luc cells and advice. The authors thank Dr. Robert Blank (Medical College of Wisconsin, Milwaukee, WI) for valuable input and advice.

Grant Support

F. Asimakopoulos is the recipient of an American Society of Hematology Bridge Grant and a Brian D. Novis grant by the International Myeloma Foundation. J.L. Jensen is a recipient of a grant from the Wisconsin Alumni Research Foundation through the UW Graduate School and a TL1 trainee award from the UW Clinical and Translational Science Award (CTSA) program (TL1TR000429: P.I. Marc Dreizen). C. Hope is the recipient of a Kirschstein National Research Service Award (T32HL007899-Hematology in Training: PI, John Sheehan) and a grant from the Wisconsin Alumni Research Foundation through the UW Graduate School. This work was supported in part by funds from the UWCCCT Trillium Fund for Multiple Myeloma Research, the UW Department of Medicine, the UW Carbone Cancer Center (Core Grant P30 CA014520), the UW-Madison School of Medicine and Public Health, the Clinical and Translational Science Award (CTSA) program, through the NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR000427, NIH-NCl grants CA87025 and CA32685, and a grant from the Midwest Athletes Against Childhood Cancer (MACC) Fund.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 20, 2015; revised April 8, 2015; accepted April 22, 2015; published OnlineFirst May 4, 2015.
20. Buhtoiarov IN, Lum H, Berke G, Paulnock DM, Sondel PM, Rakhmilevich AL.


Tumoricidal Effects of Macrophage-Activating Immunotherapy in a Murine Model of Relapsed/Refractory Multiple Myeloma


Updated version
Access the most recent version of this article at:
doi:10.1158/2326-6066.CIR-15-0025-T

Supplementary Material
Access the most recent supplemental material at:
http://cancerimmunolres.aacrjournals.org/content/suppl/2015/05/02/2326-6066.CIR-15-0025-T.DC1

Cited articles
This article cites 50 articles, 17 of which you can access for free at:
http://cancerimmunolres.aacrjournals.org/content/3/8/881.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/3/8/881.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.