Enhanced Therapeutic Efficacy and Memory of Tumor-Specific CD8 T Cells by Ex Vivo PI3K-δ Inhibition

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Abstract

Inhibition of specific Akt isoforms in CD8⁺ T cells promotes favored differentiation into memory versus effector cells, the former of which are superior in mediating antitumor immunity. In this study, we investigated the role of upstream PI3K isoforms in CD8⁺ T-cell differentiation and assessed the potential use of PI3K isom–specific inhibitors to favorably condition CD8⁺ T cells for adoptive cell therapy. The phenotype and proliferative ability of tumor antigen–specific CD8⁺ T cells was assessed in the presence of PI3K-α, -δ, or -ι inhibitors. Inhibition of PI3K-δ, but not PI3K-α or PI3K-ι, delayed terminal differentiation of CD8⁺ T cells and maintained the memory phenotype, thus enhancing their proliferative ability and survival while maintaining their cytokine and granzyme B production ability. This effect was preserved in vivo after ex vivo PI3K-δ inhibition in CD8⁺ T cells destined for adoptive transfer, enhancing their survival and also the antitumor therapeutic activity of a tumor-specific peptide vaccine. Our results outline a mechanism by which inhibitions of a single PI3K isom can enhance the proliferative potential, function, and survival of CD8⁺ T cells, with potential clinical implications for adoptive cell transfer and vaccine-based immunotherapies.

Cancer Res; 77(15); 4135-45. © 2017 AACR.

Introduction

CD8⁺ T-cell response comprises effector and memory T cells (1, 2). Memory T cells possess enhanced proliferative ability, greater functionality, and better longevity than effector cells. Different subsets of CD8 memory T cells, including effector memory (TEM) and central memory T cells (TCM), represent different stages of the CD8 differentiation spectrum (2, 3). TCM are an earlier stage of differentiation and therefore possess superior qualities, enabling them to better fight microbial challenges and mediate therapeutic antitumor immunity when compared with TEM, which in turn are superior to the terminally differentiated effector cells (4–7).

The differentiation of T cells is under the control of the PI3K/Akt pathway (1). Akt activation was found to regulate the effector/memory CD8⁺ T-cell differentiation (8). In fact, Akt inhibition was reported to augment antitumor immune responses by enhancing the expansion of CD8⁺ T cells with memory characteristics (9). Also, we have reported that inhibition of specific Akt isoforms, Akt1 and Akt2, delays the terminal differentiation of CD8⁺ T cells while enhancing the TEM phenotype. Targeting these specific Akt isoforms therefore enhanced the proliferative ability, longevity, and cytokine production in CD8⁺ T cells (10).

We therefore investigated the potential role of specific upstream PI3K isoforms in the regulation of the CD8⁺ T-cell differentiation with the aim of exploring the potential use of specific PI3K isoforms inhibitors to condition CD8⁺ T cells for adoptive cell transfer for better therapeutic outcome.

We found that the inhibition of only PI3K-δ, but not PI3K-ι or PI3K-ι, delays the terminal differentiation of CD8⁺ T-cell and maintains the memory phenotype thus enhancing their proliferative ability and survival while maintaining their cytokine and granzyme B production ability. We further demonstrated the ex vivo PI3K-δ inhibition enhances antitumor therapeutic ability of adoptively transferred CD8⁺ T cells in animal models compared with nontreated CD8⁺ T cells.

In cancer immunotherapy, it is important to maintain a CD8 that is antigen specific, highly cytotoxic, renewable, highly proliferative, and in earlier stages of differentiation to delay exhaustion. Here, we report that ex vivo inhibition of a single isoform, PI3K-δ, in CD8⁺ T cells enhances their proliferation, cytokine production, and subsequently their antitumor therapeutic ability and delays their exhaustion.

This discovery has important clinical implications. Recently, clinical trials using the PI3K-8 inhibitor idelalisib (Zydelig, CAL-101) to treat malignancies were put on hold (11) and new guidelines for its prescription were introduced. This was due to the increased number of deaths in the idelalisib (Zydelig, CAL-101) group, which was mainly attributed to infections by
P. jirovecii and cytomegalovirus, in addition to respiratory events possibly caused by infection (12). Our discovery provides a plausible explanation, where the use of PI3K-δ inhibitors delays the later stages of CD8 differentiation, which are thought to be the most potent against opportunistic viral infections. Hence, their systematic administration deprives the body of the most potent antiviral CD8+ T cells.

Our findings suggest a strategy that enhances the antitumor therapeutic efficiency of adoptive cell transfer while avoiding the adverse effects of the systemic administration of PI3K-δ inhibitors. We show that ex vivo PI3K-δ inhibition delays terminal differentiation, maintains memory phenotypes, prolongs the lifespan, and enhances the expansion of tumor-specific CD8+ T cells without affecting their cytotoxic activity. This translates into an enhanced in vivo antitumor therapeutic ability and therefore holds great clinical implications for the use of these inhibitors as immunomodulators in a safe and effective approach.

Materials and Methods
Mice and reagents
pMel-1 mice (B6.Cg-Thy1.1/Cy Tg[TcrTcrb]8Rest/J) used for in vitro experiments carry a rearranged T-cell receptor transgene (Vb13) specific for gp100 (13). For feeder cells and in vitro experiments, female C57Bl/6 (H-2b) wild-type (WT) mice were used. For in vitro experiments, 4- to 6-week-old WT female mice were used. Mice were purchased from The Jackson Laboratory and housed under pathogen-free conditions. All procedures were carried out in accordance with Institutional Animal Care and Use Committee.

The B16 cell line was purchased from ATCC, which routinely authenticates and tests cell lines (for mycoplasma, by the Hoechst stain, PCR, and the standard culture test). These cells were used in experiments after two to three passages from thawing (between 2014 and 2015). B16 was authenticated and tested for mouse parvovirus (MPV) and mouse hepatitis virus (MHV) using PCR at Augusta University. All tests were negative.

Inhibitors were purchased from Selleckchem. GDC-0941 is a pan-PI3K inhibitor with IC50 of 3 nmol/L for p110α, 33 nmol/L for p110β, 8 nmol/L for p110γ, and 0.4 nmol/L for p110δ. GDC-0941 was used in vitro at 11, 33, 99, and 279 nmol/L concentrations, ensuring inhibition of all three class 1 isoforms. A66 is a selective p110α inhibitor with IC50 of 32 nmol/L for the p110α, 236 nmol/L for PI4Kβ, 462 nmol/L for C2β, and >1.25 μmol/L for p110δ. A66 was used in vitro at 32, 96, and 288 nmol/L concentrations, ensuring selectivity to PI3Kα. TGX-221 is a highly selective PI3Kδ inhibitor with IC50 of 5 μmol/L for p110α, 5 μmol/L for p110β, 0.1 μmol/L for p110γ, and >10 μmol/L for p110δ. In vitro experiments, TGX-221 was used at 5, 15, and 45 nmol/L to ensure selectivity. CAL-101 is a selective PI3Kδ inhibitor with IC50 of 820 nmol/L for p110α, 565 nmol/L for p110β, 2.5 μmol/L for p110δ, and 89 nmol/L for p110γ. This inhibitor was tested in vitro at 0.25, 0.83, 2.5, 7.5, 22.5, 67.5, and 202.5 nmol/L to maximize the drug's specificity.

The gp10025-33 9-mer peptide (KVPVRQDQW; ANASPEC) was used for in vitro activation of pMel-1 splenocytes at 1 μmol/L as described (10). For in vivo experiments, the vaccine was prepared using the same gp10025-33 peptide and administered at 100 μg per mouse in combination with PADRE at 10 μg and Quil-A at 25 μg per mouse.

Lymphodepletion of mice was achieved using a combination of 250 mg/kg cyclophosphamide (Sigma) and 50 mg/kg fluorouracil (Selleckchem).

In vitro activation of CD8+ T cells
Tumor antigen–specific CD8+ T cells. CD8+ T cells from pMel-1 mice were activated in vitro as described (10). Briefly, homogenized pMel-1 splenocytes were stimulated with gp10025-33 peptide at 1 μmol/L (day 0). Cells were cultured in RPMI-1640 (Lonza) supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 μg/mL), 0.1% β-mercaptoethanol (Life Technologies, Invitrogen), and IL2 (100 U/mL; Peprotech) at 37°C with 5% CO2. pMel-1 cells were cultured with or without different PI3K inhibitors. The concentration of the inhibitors was maintained throughout the culture by changing the media every 48 to 72 hours.

On days 7, 14, and 21, cells were restimulated with gp10025-33 at 1 μmol/L using feeder cells [irradiated WT splenocytes, 4,000 Rads] at 1:1 ratio using the same culture conditions.

T-cell receptor stimulation and costimulation. Viable CD8+ T cells from WT mice were sorted using FACs ARIA II (BD Biosciences; purity > 99%). Cells were activated on anti-CD3–coated plates (10 μg/mL) and cultured in activation media [IL2 (100 U/mL) and anti-CD28 (2.5 μg/mL)] in the presence or absence of PI3K inhibitors for 72 hours.

Proliferation assay and phenotyping of CD8+ T cells. Cells were labeled with 5 μmol/L Violet Cell Trace (VCT) proliferation dye (Life Technologies, Invitrogen) prior to their stimulation (day 0). Proliferation of CD8+ T cells was assessed via VCT dye dilution (day 3) using LSRII SORP with HTS Flow Cytometer (BD Biosciences). Data were analyzed using Flowjo-10 (TreeStar).

Cultured cells were harvested on days 3, 7, 14, and 21 to assess their phenotype. Cells were stained with the following surface marker antibodies (BD Biosciences): APC-Cy7–labeled anti-CD8, FITC–labeled anti-Vb13, PE–labeled anti-CD62L, APC–labeled anti-CD44, PE–CF594–labeled anti-CD127, APC–labeled anti-KLRG-1, in addition to the viability stain 7AAD. All analyses were performed on viable (7AAD–), Vb13+ CD8+ T cells.

For intracellular staining, cells were stained with the fixable near-infrared Live/Dead viability stain (Life Technologies, Invitrogen), and fixed, permeabilized, and stained with APC–labeled anti-CD8, V450–labeled anti-Vb13, PE–labeled anti-CD62L and PE–CF594–labeled anti-CD44 (BD Biosciences), and FITC–labeled granzyme B (Biolegend). The analyses were performed on viable (live/dead negative), Vb13+ CD8+ T cells.

Cytometric bead array
Using the stimulation protocol above, CD8+ T cells were harvested on day 7 after the first and second stimulation. Viable (trypan blue negative) cells were coinubcated at (1:1 ratio) with 1 μmol/L gp10025-33, pulsed irradiated splenocytes (4,000 Rads) for 24 hours using the same culture conditions. Supernatants were collected and the level of IL2, TNFα, and IFNγ was assessed using the mouse Th1/Th2/Th17 Cytokine Kit BD Cytometric Bead Array (CBA) kit. Cytokine levels were collected using an LSRII SORP with HTS Flow cytometer (BD Biosciences) and analyzed using the FCAP Array Software v3.0 (BD Biosciences).
In vivo tumor treatment

C57BL/6 female mice were implanted with 400,000 B16 cells/mouse subcutaneously (s.c.) in the right flank on day 0 (B16 expresses gp100 antigen). On day 7, mice were lymphodepleted by s.c. injection of a cocktail of 250 mg/kg cyclophosphamide and 50 mg/kg fludarabine (CyFlu). On day 8, gp100-activated CD8+ T cells from pMel-1 mice cultured in the presence or absence of CAL-101 (202.5 nmol/L for 7 days as described above) were adoptively transferred intravenously (i.v.; 1 million cells per mouse). The appropriate groups were vaccinated with gp100 peptide vaccine (gp100 25-33 with PADRE and Quil A) on days 8, 15, and 22. The vaccine doses represented stimulations 2, 3, and 4 of CD8+ T cells. Animal survival and tumor growth were monitored and animals were sacrificed upon tumor ulceration or reaching the volume of 1.5 cm3 according to institutional regulations.

Statistical analysis

Statistical parameters (average values, SD, and significant differences between groups) were calculated using Microsoft Excel and GraphPad Prism. Statistical significance between groups was determined by a paired t test or one-way ANOVA with post hoc Tukey multiple comparison test (P < 0.05 was considered statistically significant).

Results

PI3K inhibition enhances the proliferative ability and survival of CD8+ T cells by preserving the memory phenotype

Memory CD8+ T cells are superior mediators of antitumor immunity than effector cells due to their greater proliferative ability (4-7, 10). Many T-cell functions are regulated by the PI3K/Akt pathway (14, 15). To test the role of PI3K in the differentiation and proliferation of CD8+ T cells, we tested the effect of the pan-PI3K inhibitor GDC-0941 (GDC) on stimulated pMel-1 CD8+ T cells activated with 1 μmol/L gp10025-33. After 3 days of stimulation, we found that GDC-treated cells consisted of a high percentage of TCM cells (CD62LhiCD44hi) in addition to TEM cells (CD62LloCD44hi), while the majority of non-GDC-treated cells were TEM. This was observed at all concentrations used (Fig. 1A). Unlike nontreated cells, the higher percentage of memory CD8+ T cells (both TCM and TEM) was maintained after the second and third stimulations (Fig. 1A). As expected, the percentage of TEM in GDC-treated cells decreased following the second and third stimulations due to the memory recall of TCM following multiple stimulations, leading to the differentiation of TEM into TCM and effector cells. However, the memory phenotype was significantly higher than the nontreated cells after each stimulation. In fact, nontreated cells virtually lost all the TCM phenotype after the second stimulation and started losing their TEM following the third stimulation in favor of the terminally differentiated phenotype (CD62LloCD44hi). This led to a significantly higher percentage of TEM in GDC-treated cells following the third stimulation in comparison with the nontreated cells.

Taken together, these data show that PI3K inhibition delays terminal differentiation and preserves a reservoir of memory cells (TCM and TEM), after several encounters with the antigen. The nontreated cells, on the other hand, lost their TCM cells, and a significant percentage of the TEM while simultaneously reaching terminal differentiation as evidenced by the significantly higher percentage of terminally differentiated effector CD8+ T cells (CD62LloCD44hi) after consecutive stimulations.

Because TEM CD8+ T cells possess a greater proliferative ability than TCM (4-7), we assessed the proliferation and expansion of CD8+ T cells under the effect of PI3K inhibitors. After 3 days of the first stimulation, the proliferation and expansion of CD8+ T cells treated with GDC was only slightly inhibited (Fig. 1B), which is expected given the role PI3K plays in the proliferation of T cells. At the highest concentration tested (279 nmol/L), the inhibitor was found to be toxic and was therefore used at lower concentrations for the rest of the experiments. However, with further stimulation (stimulations 2 and 3), CD8+ T cells treated with the PI3K inhibitor expanded at a significantly higher rate than nontreated cells (Fig. 1B). Nontreated cells lost the ability to expand following the third encounter with the antigen. These findings show that PI3K inhibition enhances the cell proliferation and survival of CD8+ T cells, which correlates with their memory phenotype.

PI3K inhibition does not affect the ability of CD8+ T cells to produce cytokotoxic cytokines and granzyme B

We have demonstrated that PI3K inhibition enhances proliferation of CD8+ T cells, preserves the TCM phenotype, and delays terminal differentiation. Classically, effector functions were thought to peak at the effector state (2); however, more recent findings suggest that memory cells are superior in their cytokotoxic abilities because of memory recall and proliferation potential, and therefore are superior options for ACT (7, 10, 16). To assess whether the function of the resultant TCM cells is affected by the inhibition of PI3K, we measured their ability to secrete IFNγ and TNFα and the level of granzyme B production.

CD8+ T cells were restimulated on days 7 and 14 (stim 2 and 3) and the level of IFNγ and TNFα production after 24 hours was assessed. After the second and third stimulations, GDC-treated and nontreated cells produced high and comparable levels of IFNγ and TNFα in response to antigen reencountering (Fig. 1D).

To further test the cytokotoxic ability of the CD8+ T cells treated with PI3K inhibitor, we assessed the level of granzyme B production by intracellular staining. As shown below, following the first and second stimulations, we found that the inhibition of PI3K does not affect the production of granzyme B when compared with CD8+ T cells that were not treated with PI3K inhibitors.

The maintained levels of IFNγ and TNFα secretion and Granzyme B production suggest that CD8+ T cells treated with PI3K inhibitors maintain their cytokotoxic functionality. Added to the marked increase in their proliferation potential and their...
PI3K-δ is the isoform responsible for terminal differentiation of CD8 T cells

We have shown that PI3K inhibition in CD8+ T cells delays their terminal differentiation, preserves TCM cells, enhances their proliferative ability while maintaining their cytokine secretion ability and prolonging their survival. The role of specific PI3K isoforms (PI3K-α, PI3K-β, and PI3K-δ) in the development, proliferation, and function of CD8+ T cells is not known. Using selective PI3K inhibitors, we next tested whether the inhibition of a single PI3K isoform would be sufficient to delay terminal differentiation of CD8.

When the phenotype of the cells was assessed after 3 days of the first stimulation, CD8+ T cells treated with inhibitors specific for PI3K-α (A66) or PI3K-β (TGX-221), there were no differences in the phenotype of CD8+ T cells from the nontreated cells (Fig. 2A). However, only when the PI3K-δ inhibitor (CAL-101) was used, CD8+ T cells displayed a phenotype similar to that observed with pan-PI3K inhibition, where there was a higher percentage of TCM cells when compared with nontreated cells (Fig. 2A; Supplementary Fig. S1). As expected, due to memory recall, this effect was less prominent after the second and third stimulations when compared with the first stimulation. Furthermore, the inhibition of PI3K-δ lead to a significantly lower percentage of terminally differentiated CD8+ T cells (CD62LloCD44lo; Fig. 2A; Supplementary Fig. S1). In particular, after the third stimulation. These findings suggest that PI3K-δ is the isoform responsible for terminal differentiation of CD8+ T cells and that its inhibition maintains CD8+ T cells in earlier stages of differentiation (both TCM and TEM) even after several encounters with the antigen.

To test if the memory phenotype generated by the inhibition of PI3K-δ possesses an enhanced proliferative ability, we assessed the proliferation of CD8+ T cells under same stimulation conditions using specific PI3K isoform inhibitors. While we found that the inhibition of PI3K-δ marginally inhibited the proliferation of CD8+ T cells compared with cells treated with PI3K-α and PI3K-β inhibitors (Fig. 2B), the inhibition of PI3K-δ, but not PI3K-α or PI3K-β significantly enhanced the proliferative ability of CD8+ T cells with further stimulations (days 7 and 14; Fig. 2C). We also found that treatment of CD8+ T cells with the PI3K-δ inhibitor maintained high expression levels of CD62L (Fig. 3A) and high secretion levels of IL2 (Fig. 3B; Supplementary Fig. S2), consistent with the enhanced proliferative ability of the memory CD8+ T cells. These high levels of CD62L expression and IL2 secretion were not observed when CD8+ T cells were treated with either PI3K-α or PI3K-β inhibitors.

Treating CD8+ T cells with the PI3K α, β, or δ isofom specific inhibitors did not affect the cells’ ability to produce TNFα and IFNγ secretion (Fig. 4A; Supplementary Fig. S2) and granzyme B (Fig. 4B). This is important as it emphasizes that maintaining the cells in the early stages of differentiation does not affect their cytotoxic ability.

Similar results were observed when PI3K-δ was silenced in CD8+ T cells, where the cells maintained a higher percentage of central memory phenotype in comparison with knocking down PI3K-α or β (Supplementary Fig. S3A). Furthermore, the proliferation of CD8+ T cells and their ability to produce granzyme B was not affected by the knockdown of any of the isoforms (Supplementary Fig. S3A).

These findings were replicated in human CD8+ T cells, where the stimulation of purified human CD8+ T cells from healthy human donors in the presence of the pan-PI3K inhibitor GDC resulted in maintaining a high level of CD62L without affecting the proliferation of the cells (Supplementary Fig. S3B). Furthermore, the inhibition of PI3K-δ in activated human CD8+ T cells resulted in the maintenance of a high expression level of CD62L, which was not observed when PI3K-α or PI3K-β were inhibited (Supplementary Fig. S3B).

Taken together, our data demonstrate that PI3K-δ is responsible for the terminal differentiation of CD8 and the inhibition of PI3K-δ, but not PI3K-α or PI3K-β, preserves CD8+ T cells in memory state, thus enhancing their proliferative potential, longevity, and survival without affecting their ability to produce cytokines and granzyme B.

The inhibition of PI3K-δ in CD8+ T cells significantly enhances their antitumor therapeutic ability in vivo

We have shown that PI3K-δ inhibition delays the terminal differentiation of CD8+ T cells and enhances their proliferative ability and survival without affecting their ability to produce cytokines and granzyme B. To test if these findings translate into enhanced therapeutic ability in vivo, we adoptively transferred tumor antigen–specific CD8+ T cells treated with CAL-101 into tumor bearing mice and assessed their antitumor effect in combination with a tumor specific vaccine.

Briefly, pMel-1 cells activated with gp100 with or without CAL-101 were cultured for 7 days, and their phenotype was assessed. Similar to what is presented above, treated cells consisted of a

Figure 1. PI3K inhibition preserves the memory phenotype and enhances the proliferative ability of CD8+ T cells. Nonfractionated splenocytes from pMel-1 mice were stained with VCT and activated with gp100 peptide (1 μmol/L) in the presence or absence of GDC-0941 (1, 35, 99, and 279 nmol/L). The cells were restimulated with gp100 peptide on days 7 and 14 and their phenotype and proliferation assessed. Gated cells were viable (7AAD-) CD8+ VCT+ A. In this representative example (left), non-GDC-treated CD8+ T cells are mainly TCM cells (CD62L+CD44+; 96%), while GDC-treated cells have a high percentage (78%) at the highest concentration (37%) of TCM phenotype (CD62L+CD44-). Terminally differentiated T cells (CD62L-CD44+; Fig. 2A) after the third stimulation are significantly higher in nontreated cells (36%) compared with only 4% with the highest GDC concentration. TCM and TEM were maintained with GDC treatment after the third stimulation (3.5% and 93%, respectively), compared with only less than 0.01 and 64% in the nontreated cells. The right panel shows bar graphs summarizing data from at least two independent experiments. **P < 0.01. After 3 days of stimulation, the proliferation of CD8+ T cells was inhibited in a dose-dependent manner by GDC-0941 (VCT dilution; far left). CD8+ T cells treated with GDC expanded at a significantly high rate with further stimulations. **P < 0.01. **P < 0.005. C, PI3K inhibition by GDC-0941 maintains a high level of CD62L expression in CD8+ T cells on day 3 and on day 7 after each stimulation with gp100. Top, a representative example of the CD62L expression. Bottom, mean fluorescence intensity (MFI) for CD62L expression (data from at least two independent experiments). **P < 0.01; **P < 0.005. D, GDC-treated CD8+ T cells secrete significantly higher levels of IFNγ following stimulation 3, which is consistent with their higher proliferative potential. Data normalized to gp100; **P < 0.05. The ability of CD8+ T cells to produce IFNγ and TNFα was not affected by PI3K inhibition using GDC-0941.
large percentage of TCM. These cells were adoptively transferred into tumor bearing, lymphodepleted mice in combination with gp100 vaccine (administered on days 8, 15, and 22 and corresponding to stimulations 2, 3, and 4; Fig. 5A).

Remarkably, the ACT of CD8⁺ T cells that were activated in vitro in the presence of the PI3K-δ inhibitor CAL-101 greatly slowed down tumor growth in B16 tumor bearing mice. This effect was significantly enhanced when the ACT was combined with the gp100 peptide vaccine because the vaccine acted a second stimulation for the CD8⁺ T cells, hence the more potent expansion, and the resulting antitumor effect. The enhanced therapeutic efficacy was much greater than any other single therapy, including the vaccine, the ACT of non–CAL-101-treated CD8⁺ T cells or the combination of both (Fig. 5B and C).

Furthermore, the combination of ACT of CAL-101–treated CD8⁺ T cells with the vaccine greatly prolonged the animal
survival (Fig. 5D). Similar results were obtained when treatment was started at a later date with larger tumors (Supplementary Fig. S4).

These data clearly demonstrate the superior antitumor functionality of CD8^+ T cells treated with a PI3K-d inhibitor. This can be attributed to the enhanced proliferative ability, longevity, survival, and maintenance of the memory phenotype.

Discussion

In response to antigen encounter, CD8^+ T-cell response comprises effector and memory T cells (1, 2). CD8 memory T cells include several subtypes, including T_{CM} and T_{EM} (2, 3). Memory cells represent earlier stages of differentiation and are superior in their cytotoxic ability against microbial challenges (4, 6) and
mediation of therapeutic antitumor immunity when compared with terminally differentiated effector cells. TCM cells are by far superior to TEM cells, due to their greater proliferative capacity upon antigen reencounter. TEM in turn are superior to the terminally differentiated effector cells (4–7).

The PI3K/Akt pathway governs many T-cell functions, including proliferation, survival, migration, and metabolism (14, 15). The differentiation of CD8 cells into memory T cells is coordinated by PI3K/Akt signaling (1, 17, 18). Continuous activation of this pathway drives the terminal differentiation, while its inhibition (at the level of Akt or the downstream mTOR) enhances the quality of CD8+ T cells by prompting a memory phenotype (8–10, 16–19).

Our group has reported that Akt1 and Akt2 isoforms are the specific drivers of terminal differentiation of CD8+ T cells and that their inhibition preserves a reservoir of highly proliferative and functionally superior memory CD8+ T cells (10).

Here, we show, for the first time, that the PI3K-δ, but not PI3K-α or PI3K-β, drives the terminal differentiation of CD8+ T cells, and that the inhibition of PI3K-δ enhances their survival

Figure 4.
PI3K inhibition does not affect the secretion of IFNγ and TNFα. CD8+ T cells from pMel-1 mice were stimulated with gp10025–33 peptide (1 μmol/L) in the presence or absence of GDC-0941 (99 nmol/L), A66 (288 nmol/L), TGX-221 (45 nmol/L), or CAL-101 (202.5 nmol/L). On days 7 and 14, CD8+ T cells were restimulated with gp10025–33 peptide and the IFNγ and TNFα levels in the supernatant assessed after 24 hours using CBA. Granzyme B expression was assessed on days 7 and 14. A, The ability of CD8+ T cells to produce IFNγ and TNFα was not affected by the inhibition of specific PI3K isoforms. B, The ability of CD8+ T cells to produce granzyme B was not affected by PI3K inhibition.
Figure 5.
The inhibition of PI3K-δ in CD8+ T cells significantly enhances their antitumor therapeutic ability in vivo. Mice were implanted with B16 in the right flank on day 0. On day 7, mice were lymphodepleted with CyFlu and on day 8, 1 million CD8+ T cells from pMel-1 mice cultured in the presence or absence of CAL-101 were adoptively transferred. The appropriate groups were vaccinated with gp100/PADRE/Quil A vaccine on days 8, 15, and 22. Animal survival and tumor growth were monitored. NT, no treatment (n = 5); Vac, vaccine (n = 5); CyFlu, cyclophosphamide/fludarabine (n = 5); CyFlu + Vac, cyclophosphamide/fludarabine + vaccine (n = 4); CD8, ACT of nontreated CD8 (n = 5); CD8 + Vac, ACT of nontreated CD8 + vaccine (n = 5); CD8/Cal, ACT of CD8 treated with CAL-101 (n = 5); CD8/Cal + Vac, ACT of CD8 treated with CAL-101 + Vaccine (n = 5). All mice that received ACT were lymphodepleted with Cy/Flu. A, Treatment schedule. B, Tumor volumes of individual mice for each treatment measured every 3 to 4 days. The data clearly show that the combination of ACT of CAL-101–treated cells with the vaccine significantly slowed down tumor growth when compared with all the other groups. C, Mean tumor volume for different groups shown in B. Statistical analyses were performed between groups on day 20 (before any of the animals died). The combination of ACT of CAL-101–treated cells with the vaccine significantly slowed down tumor growth in comparison with nontreated mice and the vaccine alone. ** P < 0.01; ****, P < 0.0001. D, The Kaplan–Meier plot depicts overall survival. The combination of ACT of CAL-101–treated cells with the vaccine significantly prolonged survival.
and proliferative ability upon reencountering the antigen by preserving a high percentage of memory CD8+ T cells. This occurs through enhancing the proliferative ability of CD8+ T cells and maintaining a high CD62L expression level and IL2 secretion. We further demonstrate that CD8+ T cells treated specifically with an inhibitor of PI3K-δ greatly enhance their antitumor therapeutic ability when adoptively transferred into tumor bearing mice.

As CD8+ T cells differentiate from naïve to effector cells, they lose their ability to produce IL2 (3). Here, we further show that PI3K-δ inhibition maintains a higher level of IL2 secretion in CD8+ T cells. Additionally, we show that the inhibition of PI3K-δ maintains a high level of CD62L, which is in agreement with these biologic functions in CD8+ T cells (2). PI3K-δ is the only isoform that controls proteolysis of CD62L is controlled by PI3K-δ downstream through Akt1 and Akt2, the two Akt isoforms we had already shown to be responsible for the differentiation of CD8+ T cells (10). We also found that in CD8+ T cells, PI3K-δ-α and PI3K-δ play no role in proliferation and survival and that these two isoforms do not signal through Akt1 and Akt2 (Supplementary Fig. S5). According, PI3K-δ is the only isoform that controls these biologic functions in CD8+ T cells.

Based on the above, our findings define a new and vital role for the PI3K-δ isofrom in T-cell biology. We demonstrate that targetting PI3K-δ can modulate the differentiation of effector and memory CD8+ T cells. This adds to the significant roles that PI3K-δ has in different T cell subsets; in particular, its definition as a key controller of the suppressive Tregs (21-23). This has important clinical implications for the use of PI3K-δ inhibitors to modulate both Tregs and CD8+ T cells.

Clinical trials using PI3K-δ inhibitors have recently been put on hold due to a significant increase in the incidence of opportunistic infection (mostly CMV). Here, our data provide a plausible explanation for this increase. The later stages of CD8 differentiation are thought to be the most potent against CMV; it is therefore apparent that the use of PI3K-δ inhibitors delays the later stages of differentiation; hence, their systematic administration could deprive the body of the most potent antiviral CD8+ T cells. We have shown this to be the case in PI3K-δ KO mice, where the percentage CD8+ T cells at later stages of differentiation in response to antigen administration (in the form of a peptide vaccine) was significantly lower in KO mice in comparison with WT mice (Supplementary Fig. S6). This also explains the findings that PI3K-δ is required for the generation of an immediate effector response to viral and intracellular bacterial infections (24, 25).

In summary, we report that PI3K-δ inhibition, but not PI3K-α or PI3K-β, enhances the memory phenotype, improves CD8+ T-cell survival, and enhances their proliferative potential while maintaining their ability to produce cytotoxic cytokines and granzyme B. These findings translate into antitumor therapeutic efficacy where the ACT of ex vivo PI3K-δ–treated CD8+ T cells in an animal tumor model greatly slows down tumor growth and prolongs animal survival.

Agents with the ability to delay terminal differentiation of CD8+ T cells without affecting their effector function and proliferation are needed. Here, we outline a strategy that enhances the memory phenotype, proliferative potential, and survival without affecting the effector function of CD8+ T cells by targeting PI3K-δ. Our findings have significant clinical implications and strongly suggest the clinical use of PI3K-δ inhibitors as potent modulators of the immune response as part of different cancer immune therapy strategies.

Disclosure of Potential Conflicts of Interest
Y. Lin is a scientist at La Jolla Institute for Allergy and Immunology. M. Mkrtichyan is a scientist at FivePrime Therapeutics Inc. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
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Acknowledgments
The authors would like to thank Dr. Frank Ward for his advice on experimental design.

Grant Support
This work was supported by grants from the Georgia Cancer Center, Augusta University to S. Khleif.

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Received July 21, 2016; revised February 10, 2017; accepted June 5, 2017; published OnlineFirst June 14, 2017.
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Enhanced Therapeutic Efficacy and Memory of Tumor-Specific CD8 T Cells by *Ex Vivo* PI3K-δ Inhibition

Rasha Abu Eid, Shamim Ahmad, Yuan Lin, et al.

*Cancer Res* 2017;77:4135-4145. Published OnlineFirst June 14, 2017.

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