Poster 1158

Preclinical Antitumor Activity of Potent and Orally Bioavailable PROTAC HPK1 Degraders as Monotherapy or Combined With an Anti-PD-1 Antibody

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Objective

- To apply a rational, iterative PROteolysis TArgeting Chimera (PROTAC) design approach to develop PROTAC hematopoietic progenitor kinase 1 (HPK1) degraders and to characterize the lead molecule, ARV-6723

Key Findings

- PROTAC HPK1 degraders demonstrated potent reduction of HPK1 levels and robust cellular pathway engagement (phosphorylation of SH2 domain-containing leukocyte protein of 76 kDa [SLP76]) and T-cell activity (interleukin-2 [IL-2]) as well as reversal of T-cell exhaustion and natural killer (NK) cell activation
- A single 30 mg/kg oral dose of ARV-6723 induced deep and sustained HPK1 degradation and efficient signaling pathway engagement in vivo
- In a highly immunogenic syngeneic colon tumor model, treatment with 30 mg/kg once daily (QD) ARV-6723 produced significantly greater tumor growth inhibition (TGI) and extended survival compared with an HPK1 inhibitor or an anti-programmed cell death protein 1 (PD-1) antibody
- Coadministration of ARV-6723 with an anti-PD-1 antibody resulted in 50% complete responders and protection from tumor rechallenge
- In a low-immunogenic melanoma model, 30 mg/kg QD ARV-6723 demonstrated superior TGI vs an HPK1 inhibitor or anti-PD-1 antibody

Conclusions

- PROTAC-induced degradation of HPK1 demonstrated greater antitumor activity than an HPK1 inhibitor or immune checkpoint blockade in preclinical models
- Combination of ARV-6723 with anti–PD-1 therapy elicited complete tumor regressions and immune memory in vivo
- These results support future investigation of ARV-6723 alone or in combination with other agents in patients with high- or low-immunogenic tumors

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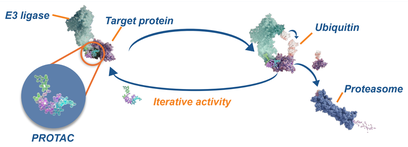
Background

- HPK1 (MAP4K1) is a serine/threonine protein kinase which, after development, is almost exclusively expressed in hematopoietic cells[1,2]
- HPK1 is a negative regulator of T cell receptor (TCR) signaling through phosphorylation of SLP76 at serine 376, proximal to the TCR, resulting in SLP76 degradation and reduced signalosome activity[3]
- HPK1 has regulatory roles in immune cell types beyond T cells (eg, B cells, NK cells, and dendritic cells) that are less well understood[4-6]
- Importantly, the HPK1 protein structure contains several proline-rich motifs suggesting a scaffolding function, which implies that it could have regulatory activities independent of its kinase function[7]
- Genetic knockout murine models have demonstrated that HPK1 loss results in enhanced immune responses (eg, T cell activation and proliferation, dendritic cell activation, and antitumor response) [5,6,8-10]
- PROTACs harness the ubiquitin-proteasome system to induce degradation of disease-causing proteins (**Figure 1**)
  - A PROTAC is a bifunctional small molecule with target protein–binding and E3 ubiquitin ligase–binding regions that forms a trimer complex to induce ubiquitination and subsequent degradation of the target protein by the proteasome
- The PROTAC mechanism of action may offer advantages over small-molecule inhibitors for targeting HPK1; for example, because HPK1 has kinase-independent functions, degradation may more potently block its immune-suppressive effects than kinase inhibition
- Using a rational and iterative PROTAC design approach, we developed and identified multiple potent and selective PROTAC HPK1 degraders; data for the lead compound, ARV-6723, are shown here

Figure 1: PROTAC mechanism of action

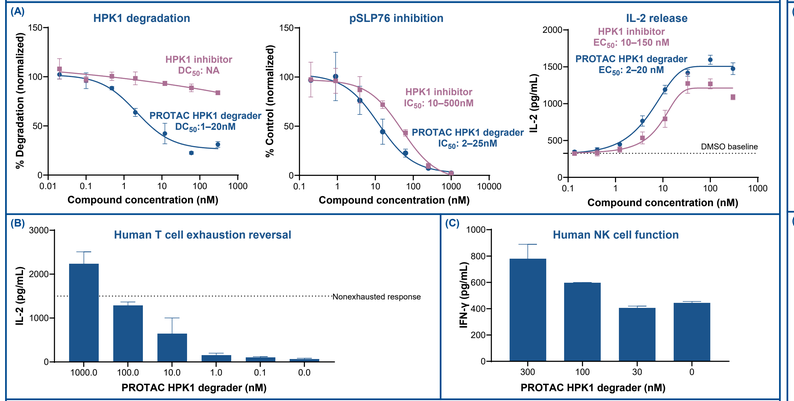


PROTAC=PROteolysis TArgeting Chimera.

Results

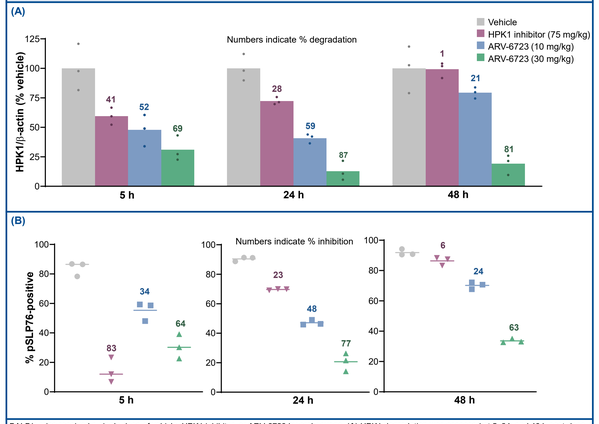
- PROTAC HPK1 degraders showed selectivity for HPK1 compared with other kinases (>500× for LCK, >50× for GLK [MAP4K3], >35× for ABL1, and >150× for Aurora A)
- Potent HPK1 degradation (half-maximal degradation concentrations of 1–20 nM), downstream pathway engagement (pSLP76 inhibition [half-maximal inhibitory concentrations: 2–25 nM]), and T-cell activity (IL-2 release [half-maximal effective concentrations: 2–20 nM]) were seen with PROTAC HPK1 degraders (**Figure 2A**)
  - PROTAC HPK1 degrader treatment counteracted tumor-microenvironment–associated T-cell dysfunction and enhanced NK-cell function (**Figure 2B**), indicating that it enhanced immune signaling beyond T cells
- ARV-6723 demonstrated deep, sustained HPK1 degradation in spleen (81% for 48 h; **Figure 3A**) and pathway inhibition (measured by pSLP76 levels; **Figure 3B**) after a single oral 30 mg/kg dose to naïve BALB/c mice
- In the highly immunogenic CT26 syngeneic tumor model, single-agent ARV-6723 administration achieved significant TGI of 57% and 88% with QD doses of 10 and 30 mg/kg, respectively, as compared with 33% and 35% for single-agent anti-PD-1 antibody and a clinical HPK1 inhibitor, respectively (**Figure 4A**)
  - ARV-6723 30 mg/kg QD significantly prolonged median survival (even after dosing discontinuation) compared with an anti-PD-1 antibody or a clinical HPK1 inhibitor (**Figure 4B**)
  - Combination of ARV-6723 with an anti-PD-1 antibody induced a high percentage of complete responders (5/10 mice with tumor volume=0 mm^3; **Figure 4C**), which were completely protected against a subsequent tumor rechallenge (**Figure 4D**) indicating immune memory
- A 30 mg/kg twice daily (BID) dose of ARV-6723 achieved significant TGI (79%) in the low-immunogenic B16F10 syngeneic tumor model, in which single-agent anti-PD-1 antibody and a 75 mg/kg BID dose of a clinical HPK1 inhibitor showed minimal effect (**Figure 5A**)
  - ARV-6723 reprogrammed immune cells in the tumor microenvironment, with significantly increased inflammatory monocytes and activated NK cells (~2-fold; **Figure 5B**) and increased (~6-fold) the number of significantly upregulated genes (over vehicle controls) as compared with an HPK1 inhibitor (**Figure 5C**)

Figure 2: In vitro pharmacology of PROTAC HPK1 degraders



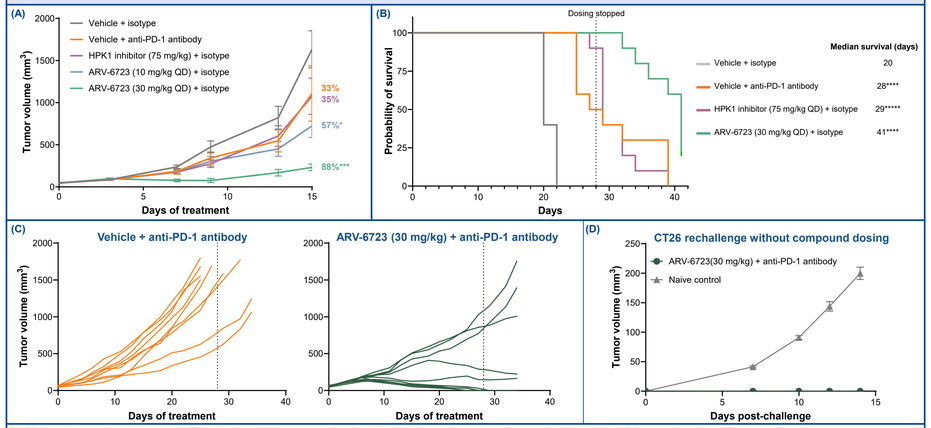
(**A**) Human PBMC and Jurkat cells were treated with PROTAC HPK1 degrader or HPK1 inhibitor for 24 h followed by measurement of HPK1 degradation and stimulation with anti-CD3/CD28 antibodies to measure pSLP76 levels and cytokine production. (**B**) Human T cells were stimulated with anti-CD3/CD28 antibodies for 7 days, rested in the presence of PROTAC HPK1 degrader or DMSO, and then restimulated with anti-CD3/CD28 antibodies for 24 h prior to measurement of secreted cytokines. (**C**) Human NK cells were isolated and expanded then treated for 24 h with PROTAC HPK1 degrader or DMSO before coculture with target K-562 cells to assess secreted cytokines. Representative data from 2-3 independent experiments with >3 donors (**A**) and 2 donors (**B** and **C**).
DC_{50}=half-maximal degradation concentration; DMSO=dimethyl sulfoxide; EC_{50}=half-maximal effective concentration; HPK1=hematopoietic progenitor kinase 1; IC_{50}=half-maximal inhibitory concentration; IFN-γ=interferon-gamma; IL-2=interleukin-2; NA=not applicable; NK=natural killer; PBMC=peripheral blood mononuclear cells; PROTAC=PROteolysis TArgeting Chimera; pSLP76=phospho-SH2 domain-containing leukocyte protein of 76 kDa.

Figure 3: In vivo PK/pharmacodynamic/target engagement time course with ARV-6723



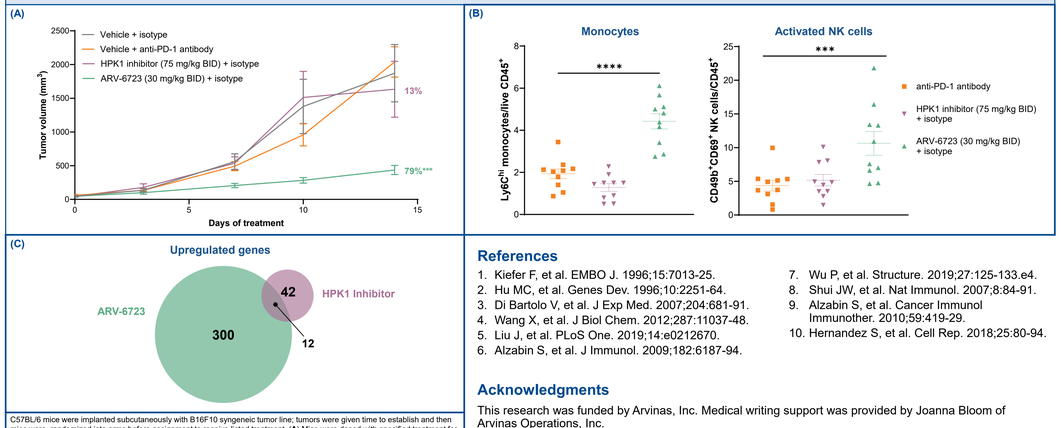
BALB/c mice received a single dose of vehicle, HPK1 inhibitor, or ARV-6723 by oral gavage. (**A**) HPK1 degradation was assessed at 5, 24, and 48 h post-dose in murine spleens via western blot with normalization to β-actin loading control. (**B**) pSLP76 levels were assessed at 5, 24, and 48 h post-dose in murine T cells isolated from lymph nodes via ex vivo stimulation with anti-CD3/CD28 antibodies.
BLQ=below the limit of quantification; HPK1=hematopoietic progenitor kinase 1; PK=pharmacokinetic; pSLP76=phospho-SH2 domain-containing leukocyte protein of 76 kDa.

Figure 4: ARV-6723 efficacy in a highly immunogenic CT26 syngeneic murine model of colon cancer



BALB/c mice were implanted subcutaneously with CT26 syngeneic tumor line; tumors were given time to establish, and then mice were randomized to receive listed treatment. (**A**) Mice were dosed with specified treatment for 15 days; tumor volumes were measured twice weekly and percentage TGI was calculated. (**B**) Mice were continually dosed with specified treatment until day 28 post-randomization when treatments were discontinued; survival was assessed using maximum allowable tumor volume as a surrogate for death and median survival was calculated. (**C**) Mice were continually dosed with specified treatment until day 28 post-randomization when treatments were discontinued; complete responders were indicated as tumor volume=0 mm^3. (**D**) Complete responders confirmed in part C were rechallenged after a 10-day washout with CT26 in the opposite flank with age-matched naïve mice as a comparator arm to assess immunological memory response. *P<0.05, ***P<0.001, and ****P<0.0001 by 1-way ANOVA.
ANOVA=analysis of variance; HPK1=hematopoietic progenitor kinase 1; PD-1=programmed cell death protein 1; QD=once daily; TGI=tumor growth inhibition.

Figure 5: ARV-6723 efficacy in a low-immunogenic and immunotherapy-resistant B16F10 melanoma model



C57BL/6 mice were implanted subcutaneously with B16F10 syngeneic tumor line; tumors were given time to establish and then mice were randomized into arms before assignment to receive listed treatment. (**A**) Mice were dosed with specified treatment for 14 days; tumor volumes were measured twice weekly and percentage tumor growth inhibition was calculated. Mice were continually dosed with specified treatment until day 7, at which point tumors were harvested, dissociated, and (**B**) immunophenotyped by flow cytometry and RNA-sequenced (**C**). ***P<0.001 by 1-way ANOVA.
ANOVA=analysis of variance; BID=twice daily; HPK1=hematopoietic progenitor kinase 1; PD-1=programmed cell death protein 1.

References

1. Kiefer F, et al. EMBO J. 1996;15:7013-25.
2. Hu MC, et al. Genes Dev. 1996;10:2251-64.
3. Di Bartolo V, et al. J Exp Med. 2007;204:681-91.
4. Wang X, et al. J Biol Chem. 2012;287:11037-48.
5. Liu J, et al. PLoS One. 2019;14:e0212670.
6. Alzabin S, et al. J Immunol. 2009;182:6187-94.
7. Wu P, et al. Structure. 2019;27:125-133.e4.
8. Shui JW, et al. Nat Immunol. 2007;8:84-91.
9. Alzabin S, et al. Cancer Immunol Immunother. 2010;59:419-29.
10. Hernandez S, et al. Cell Rep. 2018;25:80-94.

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