APL-102, an oral small molecule multi-kinase inhibitor, demonstrates favorable CSF-1R activity, offering a means for controlling tumor associated macrophages

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Background

APL-102 (previously CBT-102) is a multi-kinase inhibitor with a unique inhibitory profile. Previous presentation (Abstract #3945, AACR 2018) showed potent activity of APL-102 on VEGFR, BRAF, CRAF, key kinases in angiogenesis, MAPK pathways, and demonstrated in vivo efficacies in a broad spectrum of PDX models.

The role of the tumor microenvironment (TME) in fostering the development of malignancies is prompting the pursuit of anticancer therapies that target its components as opposed to the tumor itself. As part of their immune surveillance duties, immune cells form part of this microenvironment, and yet, cancer cells have devised means to downplay their tumoricidal capabilities. Colony stimulating factor 1 receptor (CSF-1R) may offer such a means of controlling tumor associated macrophages in the tumor microenvironment.

Materials and Methods

The potency and selectivity of APL-102 against CSF1R kinase was evaluated in a cell free system where the enzyme activity was evaluated with Eurofins standard KinaseProfiler™ system. Cell growth inhibition was evaluated in the Ba/F3 hCSF1R cell line by the CellTiter-Glo (CTG) method. Engineered cell line Ba/F3 hCSF1R was treated with APL-102 and control compounds for 3 days at 37°C, 5% CO_2 and 95% humidity.

The effect of APL-102 was further evaluated in murine myelogenous cell lines and human monocytes. Two murine cell lines M-NFS-60 and RAW264.7 were starved and then treated with APL-102 or comparator test articles for 3 days at 37°C, 5% CO_2 and 95% humidity. Human monocytes was isolated from PBMC and treated with APL-102 or control compounds for 5 days at 37°C, 5% CO_2 and 95% humidity.

In vivo efficacy studies of APL-102 in combination with an anti-PD-1 antibody were performed in two immunotherapy sensitive syngeneic models, a subcutaneous MC-38 colon model in C57BL/6J mice and a CT-26 colon model in BALB/c mice. Treatment with APL-102 and anti-PD-1 antibody as single agent and in combination were initiated when tumors reached a mean volume of 100 − 140 mm^3. Tumor volumes were measured twice weekly in two dimensions using a caliper, and the volume was calculated using the formula: V = (L × W × W)/2. Tumor growth inhibition rate (TGI%) was used to evaluate the efficacy of in vivo studies.

Results

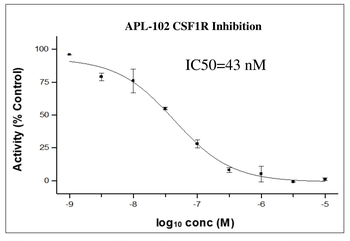


Figure 1: APL-102 inhibition of CSF1R enzyme activity was dose-dependent, with IC50 of 43 nM. The result was obtained with a radiometric enzymatic activity assay.

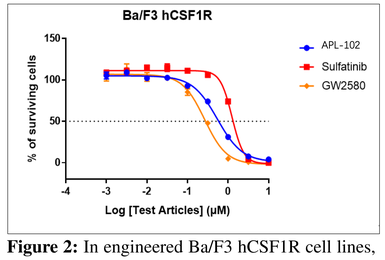
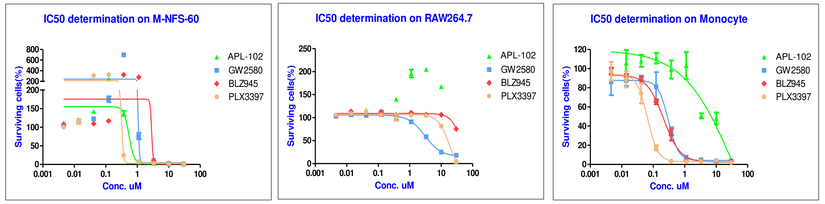


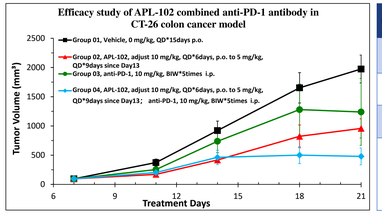
Figure 2: In engineered Ba/F3 hCSF1R cell lines, the IC50 value of APL-102 was 0.588 μM compared to 1.333 μM of sulfatinib, a similar multi-kinase inhibitor, and 0.279 μM of GW2580, a more specific CSF1R kinase inhibitor.



Cell line	APL-102		GW2580		BLZ945		PLX3397	
	IC50 (μM)	Max inh.(%)	IC50 (μM)	Max inh.(%)	IC50 (μM)	Max inh.(%)	IC50 (μM)	Max inh.(%)
M-NFS-60	**0.631**	99.44%	1.145	99.02%	3.015	99.08%	0.348	99.32%
RAW264.7	**22.85**	95.44%	4.056	81.91%	>30	24.65%	16.36	95.16%
Monocyte	**6.98**	96.05%	0.28	96.57%	0.20	96.34%	0.06	97.99%

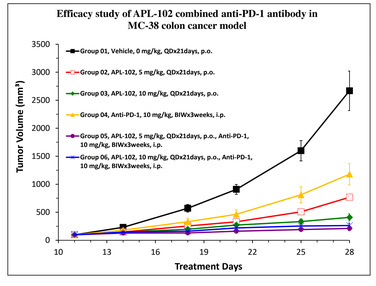
Figure 3: APL-102 demonstrated selective inhibition of the CSF-1 dependent M-NFS-60 cell line compared to CSF-1 independent RAW264.7 cell and human monocytes. In contrast, the three CSF1R targeting benchmark compounds GW2580, BLZ945, and PLX3397 showed highest inhibition of human monocyte.

Synergistic effect of APL-102 combined with anti-PD-1



Group	Treatment	Dose (mg/kg)	TV (mm^3) Day 21	TGI (%)	T/C (%)	P value
1	Vehicle	0	1974.55±239.30	--	--	--
2	APL-102	*10(5)	957.58±164.28	51.50	48.50	0.032
3	Anti-PD-1 antibody	10	1239.28±571.38	37.24	62.76	0.329
4	APL-102	*10(5)	479.07±144.00	75.74	24.26	0.001
	Anti-PD-1 antibody	10				

Figure 4: The efficacy study of APL-102 combined with an anti-PD-1 antibody in CT-26 colon cancer model. The results demonstrated effect in the combination of APL-102 with anti-PD-1 antibody. The TGI% was 75.74% in combination group, which was stronger than the single agent groups respectively.
* : Groups 2 and 4 had changed doses from 10 to 5mpk during the experiment.



Group	Treatment	Dose (mg/kg)	TV (mm^3) Day 28	TGI (%)	T/C (%)	P value
1	Vehicle	0	2668.22±353.34	--	--	--
2	APL-102	5	768.38±45.83	72.20	28.80	<0.001
3	APL-102	10	408.88±62.85	84.68	15.32	<0.001
4	Anti-PD-1 antibody	10	1180.07±190.60	55.77	44.23	0.003
5	APL-102	5	212.23±41.79	92.05	7.95	<0.001
	Anti-PD-1 antibody	10				
6	APL-102	10	263.34±42.43	90.13	9.87	<0.001
	Anti-PD-1 antibody	10				

Figure 5: Efficacy study of APL-102 combined with anti-PD-1 antibody in MC-38 colon cancer model. APL-102 showed significant inhibition on MC-38 tumor growth as a single agent. Anti-PD-1 antibody also showed inhibition as a single agent. The combination of APL-102 with anti-PD-1 antibody showed improved activity.

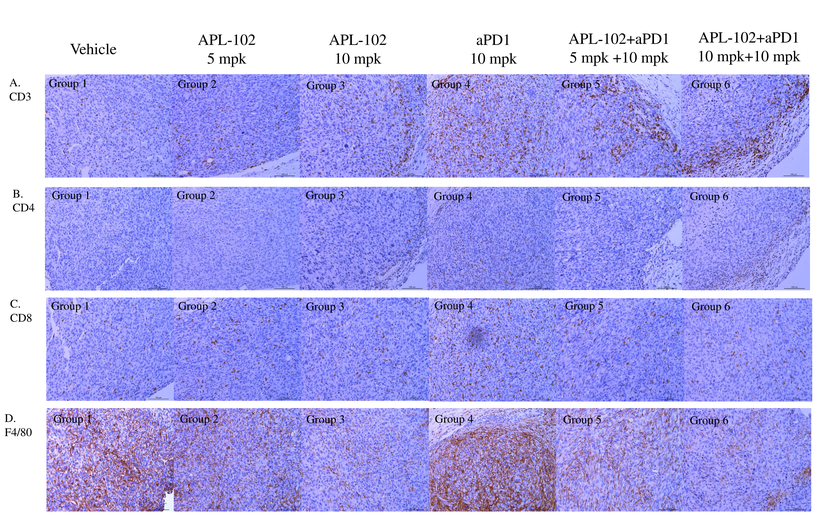


Figure 6: Expression of CD3, CD4, CD8, and F4/80 markers in tumor tissues tested by IHC in the MC-38 efficacy study. Group 1 was treated with vehicle, Group 2 was treated with 5 mg/kg of APL-102, Group 3 was treated with 10 mg/kg of APL-102, Group 4 was treated with 10 mg/kg of anti-PD-1 antibody, Group 5 was combined treatment with 5 mg/kg APL-102 and 10 mg/kg anti-PD-1 antibody, Group 6 was combined treatment with 10 mg/kg APL-102 and 10 mg/kg anti-PD-1 antibody. The results demonstrated that APL-102 increased the total T cells (CD3, Panel A), CD8 T cells (CD8, Panel C), but had minimal effect on CD4 T cells (CD4, Panel B). At the same time, APL-102 treatment significantly decreased macrophages in the tumor, both as single agent and in combination with an anti-PD-1 antibody.

Summary

The results demonstrated that APL-102 inhibits CSF1R in a radiometric enzyme activity assay with an IC50 of 43 nM. APL-102 demonstrated growth inhibition in cells dependent on CSF1-CSF1R signaling.

We demonstrated a mechanism of action for APL-102 anti-tumor activity, the inhibition of CSF1R-dependent tumor associated macrophages in TME in addition to targeting VEGFR-dependent angiogenesis and MAPK pathway. Combination of APL-102 and anti-PD-1 produced a more robust response than either single agent alone in syngeneic mouse models, which was associated with macrophage inhibition in TME. Rational combination with check-point inhibitors (CPIs) may improve the efficacy of APL-102 and broaden the efficacy of CPIs. Further studies may be needed to delineate the interplay among APL-102's different mechanisms and its synergistic effects in combination with CPIs.

Contact / Further Information

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