Tumor Regression in a Mouse Model of Hepatocellular Carcinoma Upon Treatment with the STING Agonist ALG-031048

Poster #0823

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Background

Hepatocellular Carcinoma (HCC) is the most common histological subtype of primary liver cancer in adults and the third leading cause of cancer mortality worldwide with more than 700 000 deaths per year. Most HCC patients are diagnosed at advanced stages and are not eligible for resection or transplant. The standard of care for advanced HCC patients in front line and second line therapy consists of two distinct systemic approaches: anti-angiogenic agents and immune checkpoint inhibitors. However, most patients do not respond or relapse after systemic therapies and therefore, there is a need to develop more efficacious therapies for the treatment of advanced HCC. Here, we describe the anti-tumor activity of ALG-031048, a novel STING agonist in the Hepa1-6 HCC mouse model.

ALG-031048 has nanomolar potency and high in vitro stability

ALG-031048 binds to the STING isoform R232 in vitro and induces signaling in the HEK293T R232 reporter cell line with nanomolar EC_{50}. ALG-031048 has high in vitro stability with a t1/2 of > 120 min in the Ectonucleotide Pyrophosphatase Phosphodiesterase I (ENPP1); 100% of ALG-031048 can be detected after exposure to high concentrations of the snake venom phosphodiesterase (SVPD) for 24 hours. ALG-031048 is stable in biological matrices such as mouse and human liver microsomes and plasma.

Table 1: In vitro profile of ALG-031048.

	2'3' cGAMP	ADU-S100	ALG-031048
Thermal Shift Assay K_d [µM] / shift [°C]	2.75 ± 0.71 µM 16.4 °C	4.39 ± 0.50 µM 10.4 °C	3.03 ± 0.24 µM 12.8°C
HEK 293T R232 reporter assay			
EC_{50} [µM] IRF reporter	0.024 ± 0.009	0.09 ± 0.034	0.029 ± 0.017
EC_{50} [µM] IFN-β reporter	0.057 ± 0.040	0.22 ± 0.17	0.056 ± 0.028
Phosphodiesterase stability			
ENPP1 $t_{1/2}$ [min]	< 30	68	>120
SVPD % remaining after 24hrs	0%	0%	100%
Microsome stab. mouse/human $t_{1/2}$ [min]	7.2/12.8	>60/>60	>60/>60
Plasma stab. mouse/human $t_{1/2}$ [min]	>480/>480	>480/>480	>480/>480

ALG-031048 causes strong tumor regression in Hepa1-6 mouse HCC model

Dosing Hepa1-6 bearing female C57BL/6 mice with three 25 µg doses of ALG-031048 intratumorally prevented tumor growth while 100 µg ALG-031048 resulted in a mean tumor regression of 88% (***Figure 1***). 7 out 10 animals in the 25 µg and 10 out 10 animals in the 100 µg had TR of > 80%. 40 and 60% of ALG-031048 treated animals demonstrated a complete response as defined by a TV < 10 mm^3. In contrast, treatment with an anti-PD-1 antibody (10 mg/kg IP BIW, clone RMP1-14) resulted only in a mean tumor growth inhibition of 72.4%. The reduction in tumor volume was confirmed by the tumor weight at the end of the study. In line with the mechanism of action, treatment with ALG-031048 resulted in a dose-dependent increases in plasma cytokines such as interferon-β1, interferon-γ, IL-6, MIP-1α, MCP-1 and TNF-α (***Figure 2***) as well as IP-10, and IL-12(p40) (not shown).

Figure 1: Tumor reduction in Hepa1-6 bearing mice. ***black***: vehicle control; ***red:*** 10 mg/kg anti-PD-1 antibody, IP, BIW; ***light blue:*** 3 IT doses of 25 µg ALG-031048; ***dark blue***: 3 IT doses of 100 µg ALG-031048; ***main panel***: tumor volume over time; ***insert***: tumor weight on last day of study.

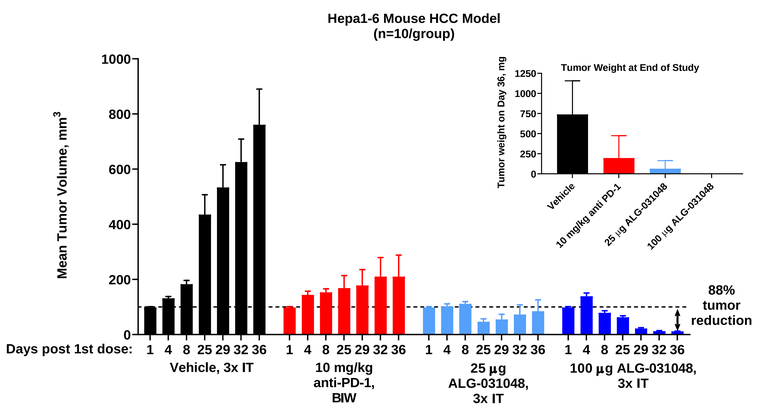
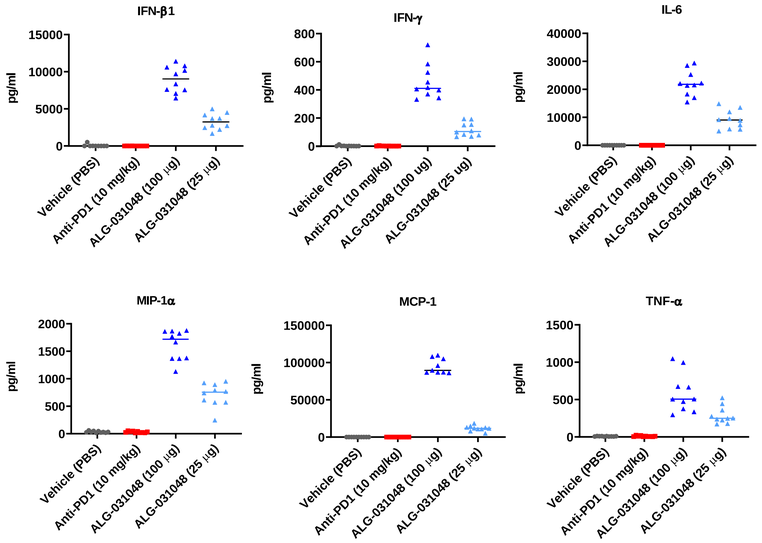


Figure 2: Plasma cytokine levels in Hepa1-6 bearing mice 6 hours after the 1st dose.



ALG-031048 demonstrates anti-tumor activity after subcutaneous dosing

Dosing CT26 tumor-bearing mice subcutaneously (SC) with 1 mg/kg ALG-031048 (3x q3d) delayed tumor growth by 4.5 days compared to the vehicle control group and to a similar extend as an approximately equivalent dose (25 µg) administered IT (***Figure 3*** and ***Table 1***). Increasing the SC dose to 4 mg/kg further delayed tumor growth to 15.4 days compared to vehicle; one mouse in this group achieved complete response (CR, TV < 10 mm^3) at the end of the study. These results provide early proof of concept that SC administration of ALG-031048 can achieve complete tumor regression. Additional studies optimizing the dosing regimen are ongoing.

Figure 3: CT26 Tumor growth curves. ***grey***: vehicle control; ***light blue:*** 3 IT doses of 25 µg ALG-031048; light green: 3 SC doses of 1 mg/kg ALG-031048; ***dark blue***: 2 IT doses of 100 µg ALG-031048; ***dark green***: 3 SC doses of 4 mg/kg ALG-031048

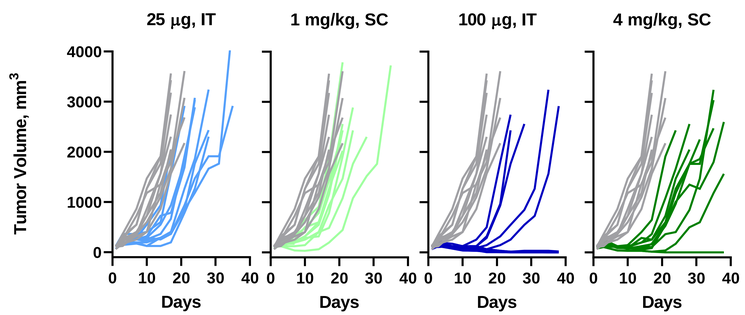


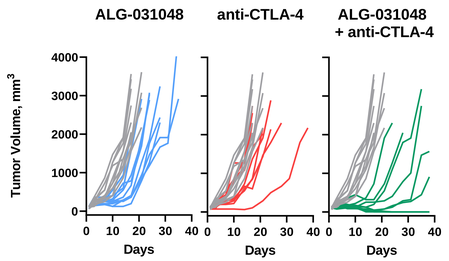
Table 1: Anti-tumoral activity of ALG-031048 upon intratumoral or subcutaneous dosing

Dose/ Route (n=10/group)	CR (TV < 10 mm^3)	Time to Endpoint (Days)
Vehicle, SC	0 %	16.4
25 µg IT	0 %	23.3
100 µg IT	40 %	40.6
1 mg/kg SC	0 %	20.9
4 mg/kg SC	10 %	31.8

Combination of ALG-031048 with checkpoint inhibitor anti-CTLA-4 improves in vivo activity

An improved anti-tumoral activity was observed when ALG-031048 was co-administered with the immune checkpoint inhibitor anti-CTLA-4. 40% of animals receiving the combination treatment demonstrated a complete response (CR), indicating a synergistic effect.

Figure 4 and Table 2: CT26 tumor-bearing mice were treated with 25 µg ALG-031048 IT (***blue***) or anti-CTLA-4 (***red***) intraperitoneally (IP) or a combination of ALG-031048 and antiCTLA-4 (***green***). The anti-CTLA-4 dosing regimen was 5 mg/kg on day 1, and 1 mg/kg on days 4 and 7. Vehicle control treated animals in ***grey***.

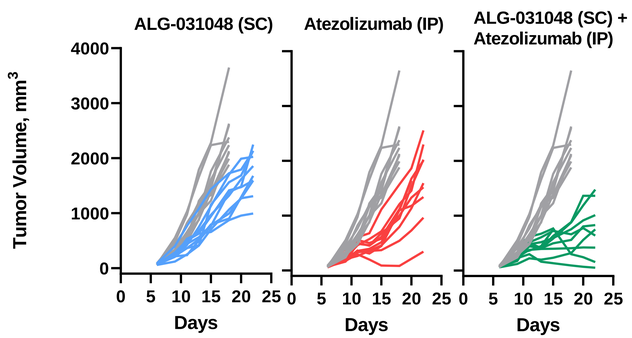


Drug n=10/group	CR (TV < 10 mm^3)	Time to Endpoint (Days)
Vehicle	0 %	16.4
25 µg ALG-031048	0 %	23.3
5/1 mg/kg anti-CTLA-4	0%	20.5
anti-CTLA-4 + ALG-031048	40%	28.6

Subcutaneous dosing of ALG-031048 improves anti-tumoral activity of atezolizumab in vivo

Subcutaneous dosing of ALG-031048 together with the anti-PDL-1 antibody atezolizumab (dosed IP) resulted in improved anti-tumoral activity in the MC38 mouse model expressing the human PD-L1 protein. Weekly SC dosing of 0.5 mg/ml ALG-031048 resulted in an average tumor growth inhibition (TGI) of 46% (day 18), while a biweekly IP dose of 5 mg/kg atezolizumab lead to a TGI of 60% (***Figure 5***). The combination of ALG-031048 with atezolizumab further improved the TGI to 77%. A tumor reduction was observed in one animal.

Figure 5: MC38-hPD-L1 tumor-bearing mice were treated with 0.5 mg/kg ALG-031048 SC (3x QW) (***blue***) or 5 mg/kg Atezolizumab IP (6x BIW) (***red***) or a combination of ALG-031048 and atezolizumab (***green***). Vehicle control treated animals in ***grey***. Dosing started when animals reached an average TV of 70 mm^3 and was followed for up to 22 days.



Summary:

- ALG-031048 dosed intratumorally induced a robust anti-tumor response in the Hepa1-6 mouse model of HCC, resulting in complete tumor regression in 60% of the animals
  - Consistent with the MOA, an increase in circulating type I interferons and pro-inflammatory cytokines was observed
  - In the Hepa1-6 HCC model, ALG-031048 was more efficacious than an anti-PD-1 antibody
- The strong anti-tumoral effect of ALG-031048 was confirmed in the CT-26 and B16-F10 model (not shown)
- ALG-031048 demonstrated anti-tumoral activity after subcutaneous administration
- ALG-031048 improves the antitumor efficacy of immune checkpoint inhibitors anti-CTLA-4 and anti-PD-L1

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