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RGX-202, a first-in-class small molecule inhibitor of the creatine transporter SLC6a8, is a robust suppressor of cancer growth and metastatic progression

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INTRODUCTION

Creatine metabolism generates high energy phosphates

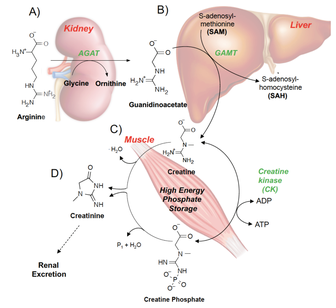
A) Guanidinoacetate (GAA) is formed through the action of arginine:glycine amidinotransferase (AGAT) on arginine and glycine.

B) In the liver, creatine is formed through methylation of GAA by guanidino-acetate N-methyltransferase (GAMT).

C) Active uptake by the creatine transporter SLC6a8 then supplies creatine to organs of high fluctuating energy demands where it is phosphorylated by creatine kinases (CK) to generate phosphocreatine (p-Cr). p-Cr serves as a high energy metabolite to be used in various ATP-requiring processes.

D) Creatine and p-Cr spontaneously convert into creatinine, which is renally excreted.

AGAT and GAMT levels are highest in the liver, kidney and pancreas. Lower expression levels have been detected in other organs including the brain, gastrointestinal tract and reproductive organs.



Metastatic colon cancer depends on creatine metabolism to fuel survival

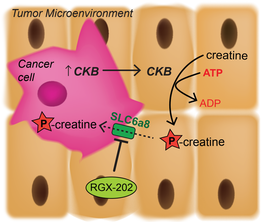
• Primary and metastatic cancer cells depend on energy sources to fuel proliferation and cancer progression. Metastatic colon cancer cells upregulate CKB and release the enzyme into the extracellular matrix where it converts available creatine and ATP into phosphocreatine that is then taken up by the cancer cell via the creatine transporter SLC6a8.

• High SLC6a8 expression in tumors is associated with reduced survival in patients with cancer[1].

• Amplifications of SLC6a8 and CKB were observed in various cancer types, including head and neck, stomach, esophageal, cervical, ovarian, lung adenocarcinoma and prostate cancer (CBioportal).

• SLC6a8 inactivation is tolerated in normal cells and gives rise to adult animals, while inactivation of this pathway causes cancer cell death[1, 2].

• Inhibition of creatine metabolism by an orally administered, first-in-class SLC6a8 inhibitor, RGX-202, robustly reduces intracellular creatine pools. This leads to reduced cell survival and induction of apoptosis causing tumor growth inhibition and tumor regressions in animal models.

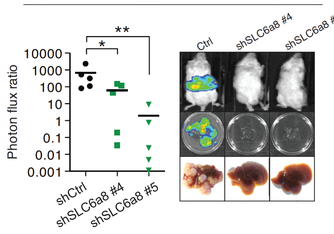


Adapted from Loo et al, Cell 2015

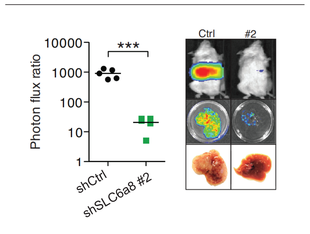
Lorem ipsum

Depletion of SLC6a8 suppresses colon cancer progression in mice

Lvm3b KRAS^{G12D} human colon cancer



SW480 KRAS^{G12V} human colon cancer



Liver colonization after splenic injection of cells +shRNA targeting SLC6a8

Loo et al., Cell 2015

RGX-202: First-in-class small-molecule SLC6a8 inhibitor

• RGX-202 is a first-in-class small-molecule SLC6a8 inhibitor.

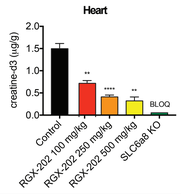
• Robustly reduces intracellular creatine and ATP pools in cancer cells.

• Rgenix owns composition of matter patent protection on RGX-202 until 2036.

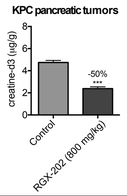
RGX-202 characteristics	
LogP	-2.8
Caco-2 permeability	Low
PgP substrate	no
% Protein bound (human)	40%
% Protein bound (mouse)	33%
% Protein bound (dog)	36%
Water Solubility	> 100 mg/mL

RGX-202 robustly inhibits SLC6a8 in vivo

Accumulation of labeled creatine-d3 in the heart following one dose of RGX-202 i.p.



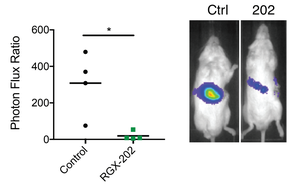
Accumulation of labeled creatine-d3 in tumors of animals receiving RGX-202, supplemented in the diet for 47 days



RGX-202 suppresses liver metastatic colonization in mice

Lvm3b KRAS^{G12D} human colon cancer

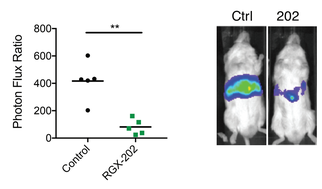
Liver colonization after splenic injection of cells + RGX-202 650 mg/kg i.p. QD



• 94% inhibition of liver colonization by Lvm3b cancer cells

PANC1 KRAS^{G12D} p53 mut human pancreatic cancer

Liver colonization after splenic injection of cells pre-treated with 10 mM RGX-202 for 48h



• 80% inhibition of liver colonization by PANC1 cancer cells

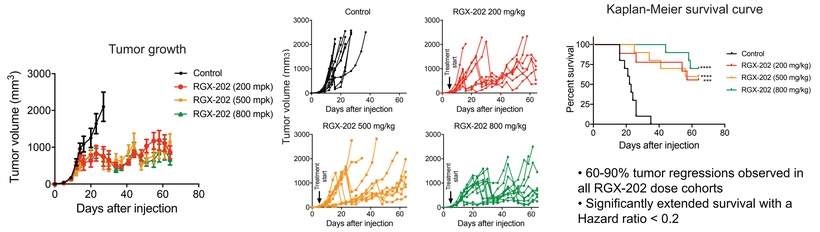
Cancer cells express a luciferase reporter gene to allow visualization of liver colonization by whole-body bioluminescence measurement

Anti-tumor efficacy of RGX-202 in human KRAS mutant xenograft models

Lvm3b KRAS^{G12D} human colon cancer

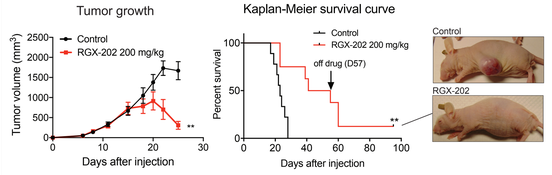
Primary tumor growth; RGX-202 treatment started when tumors reached ~30 mm3

Sustained administration (supplemented in the diet)

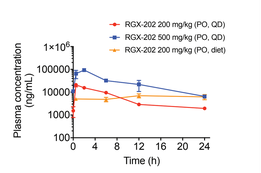


• 60-90% tumor regressions observed in all RGX-202 dose cohorts
• Significantly extended survival with a Hazard ratio < 0.2

Oral gavage administration



RGX-202 exposure in tumor bearing mice

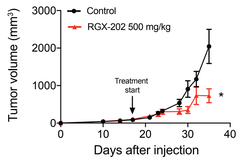


• 70% tumor growth inhibition
• 60% tumor regressions
• Hazard ratio = 0.14

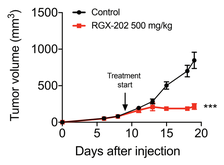
* p< 0.05
** p< 0.01
*** p< 0.001

Anti-tumor efficacy of RGX-202 in syngeneic models

CT26 KRAS^{G12D} mouse colon cancer



MC38 KRAS wild-type mouse colon cancer

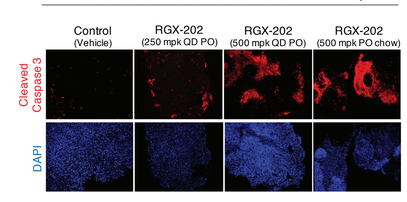


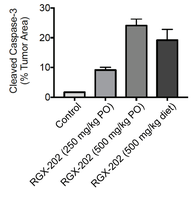
RGX-202 was administered at a dose of 500 mg/kg/day, supplemented in the diet. Treatment started when tumors reached ~100 mm^3 (CT26) or ~150 mm^3 (MC38).

RGX-202 induces tumor cell apoptosis in vivo

MC38 KRAS wild-type mouse colon cancer

Treatment started when tumors reached ~80 mm^3 for 9 days.

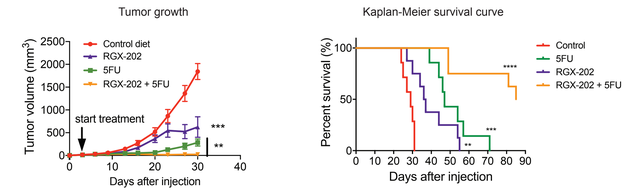




RGX-202 demonstrates additive activity with chemotherapy

CT26 KRAS^{G12D} mouse colon cancer

RGX-202 was administered at a dose of 800 mg/kg/day, supplemented in the diet. 5-FU was administered i.p. 50 mg/kg/wk

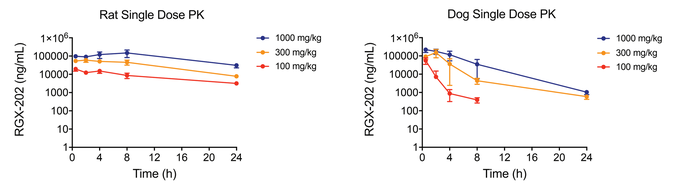


• 66% tumor growth inhibition with RGX-202 treatment; 85% tumor growth inhibition with 5-FU treatment
• 99% tumor growth inhibition in the combination cohort
• 50% complete regressions in the combination cohort
• Hazard ratio for combination cohort <0.15

Single dose pharmacokinetics

Species	n/Sex	Dose (mg/kg)	AUC$_{0\text{-}24h}$ (ng-h/mL)	C$_{max}$ (ng/mL)	T$_{max}$ (h)
Rat	3/Male	100	199,162	19,633	0.5
Rat	3/Male	300	830,332	62,167	1.5
Rat	3/Male	1000	2,355,908	149,433	5.5
Dog	4/Male	100	44,078*	44,318	0.875
Dog	4/Male	300	531,666	161,325	1.625
Dog	4/Male	1000	1,242,177	230,000	0.875

*AUC$_{0\text{-}8h}$



CLINICAL DEVELOPMENT PLAN

Rgenix is planning to open a First-in-Human study in patients with advanced gastrointestinal malignancies to study RGX-202 in a monotherapy dose escalation format. This will be followed by expansion cohorts in patients with select gastrointestinal malignancies both with RGX-202 as a monotherapy and in combination with cytotoxic chemotherapy.

CONCLUSIONS

• The creatine pathway is a critical regulator of colon cancer progression.

• Inhibition of the creatine transporter SLC6a8 by the first-in-class small molecule RGX-202, causes reduction of intracellular creatine levels and results in tumor growth inhibition and tumor regressions in animal models.

• Tumor growth inhibition is, at least partially, due to induction of intratumoral apoptosis.

• Antitumor activity has been demonstrated with RGX-202 monotherapy in several syngeneic and xenograft tumor models, including KRAS wild-type and KRAS mutant colon cancer and pancreatic cancer.

• RGX-202 treatment demonstrates additive efficacy with 5-FU chemotherapy.

• Single dose PK demonstrate close to dose-proportional exposure to RGX-202.

• Future clinical development plans include combination therapy with a cytotoxic chemotheray as well as assessment of clinical response in disease specific monotherapy and combination therapy cohorts.

• Therefore, RGX-202 represents an orally administered first-in-class compound for clinical studies of patients with GI malignancies.

REFERENCES

1. Loo JM, Scherl A, Nguyen A, Man FY, Weinberg E, Zeng Z, Saltz L, Paty PB, Tavazoie SF. Extracellular metabolic energetics can promote cancer progression. Cell. 2015; 160 (3): 393-406.
2. Skelton MR, Schaefer TL, Graham DL, deGrauw TJ, Clark JF, Williams MT, Vorhees CV. Creatine Transporter (CrT; Slc6a8) Knockout Mice as a Model of Human CrT. Plos one. 2011; 6(1):e16187-e16187.

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