Immunotherapeutic effects of the TYK2 inhibitor SAR-20351 in syngeneic tumor models

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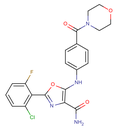
Abstract

Background: TYK2 (tyrosine kinase 2) is a member of the Janus family of non-receptor tyrosine kinases and has been shown to play an important role in the signalling of type I interferons, as well as IL-12 and IL-23, via phosphorylation of downstream STATs. The TYK2/STAT1/BCL-2 pathway is implicated in the survival of leukemic cells in a proportion of T-ALL cases[1]. It has been reported that STAT3 signalling in both the tumor and microenvironment is critical in shifting the balance from IL-12, a central cytokine in antitumor and antiviral immunity, to potentially pro-carcinogenic IL-23 production[2]. Furthermore there is increasing evidence that chronic tumor interferon signalling leads to multigenic T cell exhaustion and resistance to immune checkpoint blockade[3]. Additionally, TYK2 has been suggested to play a key role in CTLA-4 STAT3 signal transduction in B cell lymphomas and in melanoma associated B cells[4].
We have previously reported[5] on SAR-20347, a 1,3-oxazole-4 carboxamide, which is an orally bioavailable potent and selective inhibitor of TYK2, which causes tumor regression in in vivo models of T-ALL, and which has shown striking reductions in STAT phosphorylation downstream of IFNα signalling, and IFNγ production in response to IL-12 both in vitro and in vivo. Here we report the effects of SAR-20351, an orally bioavailable optimised analog of SAR-20347, on tumor cell viability and components of the tumor microenvironment in immunocompetent mouse models.
Methods: A range of syngeneic tumor models were used to establish SAR-20351 efficacy as both a monotherapy and in combination with standards of care. FACS analysis was used to identify immune cell sub-populations within tumor tissue and measure PD-1 and PD-L1 expression levels on appropriate cell types.
Results: Reduced tumor growth was observed following SAR-20351 treatment as a monotherapy in the PancO2, CT26, MC38, B16F10, Renca and A20 models, and in combination with 5-FU or anti-CTLA-4 in the colon CT26 model. Similar effects were seen in the MC38 model when SAR-20351 was combined with 5-FU and in the Renca model when SAR-20351 was combined with everolimus.
To elucidate mechanism of action in these models, tumors were grown in immunocompetent and immunodeficient animals and efficacy compared. Increased efficacy of SAR-20351 in an immunocompetent background compared to immunodeficient animals indicated immunotherapy as a mechanism of action. FACS analysis identified reduced myeloid and Treg cell infiltration in tumor tissue and reduced PD-1 expression was observed on TIL and TAMs, indicative of a less-exhausted phenotype. SAR-20351 was seen to reduce levels of PD-L1 expression by tumor cells, and reversed the increase in PD-L1 levels induced by certain chemotherapy or targeted agents. Reduction of pTYK2, pSTAT3 and cMYC was observed in B cells derived from the A20 model.
No changes in animal bodyweight or behavior, and no significant differences in complete blood counts and blood chemistry parameters, following treatment with SAR-20351 demonstrated that treatment was well-tolerated during these studies.

Conclusions: The TYK2 inhibitor, SAR-20351 results in significant control of solid tumor growth and the mechanism of action involves immunotherapy, with reductions in myeloid and Treg cell infiltration in a range of tumor models.

Introduction

We have developed a series of 1,3-oxazole-4 carboxamide analogs, exemplified by SAR-20347, which potently inhibit TYK2 with excellent selectivity against other JAK family kinases and the broader kinome. SAR-20351 is an optimised analog of SAR-20347 with improved potency and selectivity against the JAK family and broader kinome. Both SAR-20347 and SAR-20351 have shown striking reductions in disease severity in a number of models of autoimmune disorders, including psoriasis, rheumatoid arthritis and ulcerative colitis.



^{33}P-ATP [ATP] = 100μM	SAR-20347	SAR-20351
TYK2 IC_{50} nM	6	3
JAK1 IC_{50} (nM)	48	29
JAK2 IC_{50} (nM)	87	91
JAK3 IC_{50} (nM)	210	243

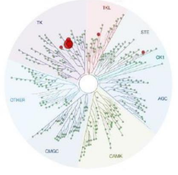


Figure 1 and Table 1: Structure of SAR-20347. JAK selectivity profile of SAR-20347 and SAR-20351 in biochemical assay. Kinome selectivity profile of SAR-20351.

Effect of SAR-20351 on cytokine signalling in human cells and mouse and human whole blood:

The ability of SAR-20351 to inhibit signalling downstream of cytokine stimulation, including IL-12, IL-23 and IFNα was examined in human cells and mouse and human whole blood assays:

	IL-23		IL-22
	pSTAT3	IL-17F	pSTAT3
SAR-20351 IC_{50} (nM)	61	203	53

Table 2: Inhibition (IC_{50}) of STAT3 phosphorylation by SAR-20351 following IL-23 or IL-22 stimulation, and IL-17F production following IL-23 stimulation. IL-23 assay performed in fresh human peripheral CD4CD45RO+ cells expanded under Th17 skewing conditions for 11 days. IL-22 assay performed in HT-29 cells. IL-17F and STAT phosphorylation was measured by ELISA

SAR-20351 inhibits cytokine signalling pathways dependent upon TYK2 and JAK1 in whole blood, with excellent selectivity over JAK2 mediated signalling:

JAKs	TYK2/JAK2	JAK1/JAK2/TYK2	JAK1/TYK2	JAK1/JAK2	JAK1/JAK3	JAK2/JAK2
Cytokine	IL-12	IL-6	IFNα	IFNγ	IL-15	GM-CSF
STAT	STAT4	STAT1	STAT1	STAT1	STAT5	STAT5
Cell Type	$CD3^+$ T	$CD3^+$ T	$CD3^+$ T	Monocyte	$CD3^+CD8^+$ T	Monocyte
Mouse	99	3332	814	349	918	>10,000
Human	145	418	167	257	539	>10,000

Table 3: Inhibition of STAT phosphorylation (IC_{50} in nM) following cytokine stimulation of mouse (DBA1/J) or human whole blood. Fresh blood was incubated with compound for 1h prior to stimulation with cytokine and staining for cell type. % reduction in STAT phosphorylation vs control was analysed by flow cytometry.

Effect of SAR-20351 on PANC02 tumor volumes and STAT protein phosphorylation levels:

PANC02 cells were injected into the rear dorsum of male C57BL/6 or male athymic nude mice. Tumors were allowed to grow to 80-120mm^3 before treatment with vehicle or SAR-20351 (25mg/kg PO bid) for 21 days. At the end of treatment tumors were removed from C57BL/6 mice for flow cytometry and western blot analyses.

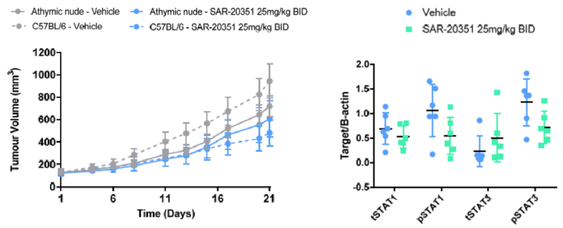


Figure 2: Volume of PANC02 tumors implanted subcutaneously in male athymic nude mice and male C57BL/6 mice treated with SAR-20351 (25 mg/kg PO bid). Values shown are mean ±SD; n=12 for all groups.

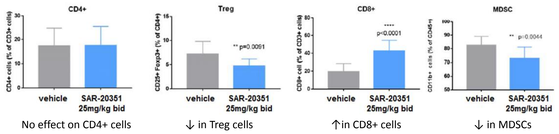
Figure 3: Densitometry readings for target proteins from excised tumors. Values were normalised by dividing each by the β-actin value for the appropriate sample.

PANC02 tumor growth rates were significantly reduced in immunocompetent C57BL/6 mice while minimal change was observed in the immunodeficient athymic nude mice. STAT1 and STAT3 phosphorylation levels were reduced but did not reach statistical significance. Animals were culled 1h after final dose.
Pharmacokinetic analysis showed that plasma and tumor levels of SAR-20351 were not significantly different between strains:

Treatment	Mouse strain	Dose (mg/kg)	Tumor concentration		Plasma concentration	
			Mean (ng/g)	SD	Mean (mg/ml)	SD
SAR-20351	Athymic nude	25 PO bid	763	433	1120	466
SAR-20351	C57bl/6	25 PO bid	899	226	1189	303

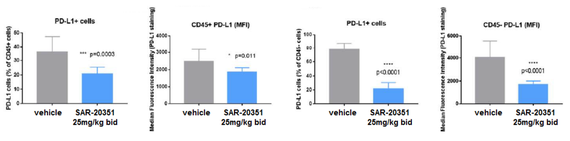
Table 4 – Mean plasma and tumor concentrations of SAR-20351 1h after the final dose (total 21-day dosing period)

Effect of SAR-20351 (25mg/kg bid) on CD4+, Treg, CD8+ and MDSC populations in PANC02 tumors:



No effect on CD4+ cells ↓ in Treg cells ↑in CD8+ cells ↓ in MDSCs

Effect of SAR-20351 (25mg/kg bid) on PD-L1 expression on CD45+ & CD45- cells in PANC02 tumors:



↓ in PD-L1 expressing cells ↓ intensity of PD-L1 staining ↓ in PD-L1 expressing cells ↓ intensity of PD-L1 staining

The effect of SAR-20351 treatment was evaluated in further syngeneic models of colorectal cancer (MC38, CT26), renal cell carcinoma (Renca) and melanoma (B16F10). Tumors were well established (100-150mm^3) prior to treatment with SAR-20351, dosed orally twice daily as monotherapy, or in combination with a SoC chemotherapy (5-FU for CT26 and MC38, everolimus for Renca) or aCTLA-4 antibody (CT26).

Effect of SAR-20351 on MC38 tumor volumes as monotherapy and in combination with 5-FU

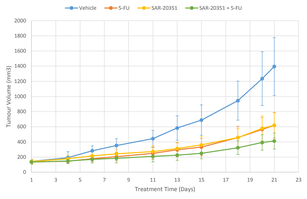


Figure 4: Volume of MC38 tumors implanted subcutaneously in C57BL/6 mice treated with SAR-20351 (50mg/kg PO bid) alone or in combination with 5-FU (40mg/kg IP QoD – total of 3 doses) for 21 days

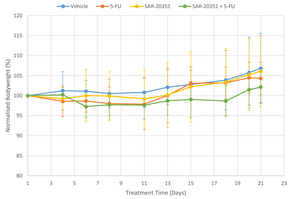


Figure 5: Normalised bodyweight of male C57BL/6 mice bearing MC38 tumors treated with SAR-20351 alone or in combination with 5-FU

SAR-20351 dosed as monotherapy and in combination with 5-FU was well-tolerated over the 21 day treatment period. Treatment with SAR-20351 50mg/kg bid alone and in combination with 5-FU resulted in significantly smaller tumor volumes compared to vehicle control (p<0.0001 for both groups. ANOVA with Tukey's post-hoc test)

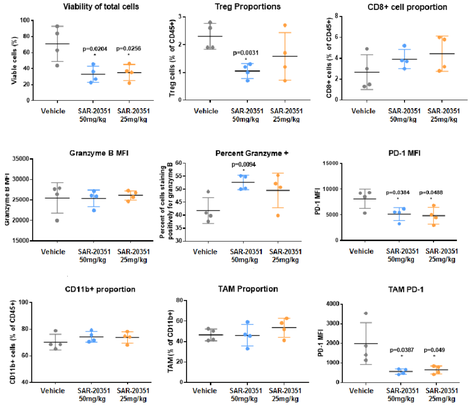
Model	Mouse strain	Treatment	Dose (mg/kg)	Tumor size (T/C) Monotherapy	Tumor size (T/C) Combination
CT 26 (Colorectal)	Balb/c	5-FU	40 IP QoD	69%	43%
		SAR-20351	50 PO bid	73%	
		aCTLA-4	0.1 IP biw	40%	27%
MC38 (Colorectal)	C57BL/6	5-FU	40 IP QoD	39%	22%
		SAR-20351	50 PO bid	38%	
Renca (Kidney)	Balb/c	Everolimus	0.25 PO QD	53%	32%
		SAR-20351	50 PO bid	43%	
B16F10 (Melanoma)	C57BL/6	SAR-20351	25 PO bid	83%	-
		SAR-20351	50 PO bid	50%	

Table 5: T/C values for SAR-20351 dosed as monotherapy and in combination with 5-FU, everolimus or aCTLA4 in syngeneic models of melanoma and colorectal and kidney cancer.

SAR-20351 was well-tolerated as monotherapy and in combination over the treatment periods. Complete blood counts revealed no additional haematological toxicity in the combinations.

FACS Analysis of MC38 tumor tissue following SAR-20351 monotherapy

Male C57BL/6 mice were implanted with MC38 tumor cells. When tumors reached 80-100mm3 treatment with SAR-20351 (25mg/kg or 50mg/kg PO bid) began for 10 days. Tumors were removed 1h after final dose and tissue digested prior to staining for flow cytometry. Samples were run through a FACS Canto II apparatus and analysis carried out using Flowjo software:



Effect of SAR-20351 on A20 tumors as monotherapy

A20 cells were injected into the rear dorsum of male Balb/c mice. When tumors reached approximately 50-100mm3 mice were randomly assigned to vehicle or treatment group. Animals in the main treatment group were dosed with SAR-20351 at 50mg/kg PO bid for 21 days. Satellite animals were dosed for 5 days and sampled for FACS analysis.

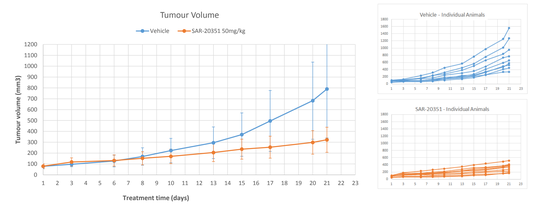
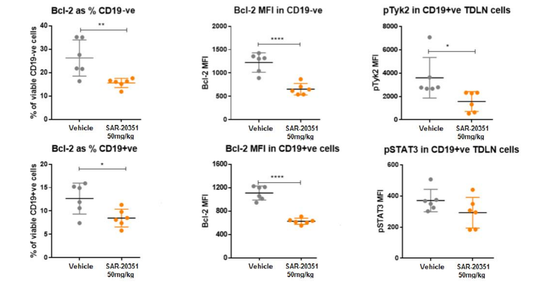


Figure 6: Average volume and individual animal data for A20 tumors implanted subcutaneously in male Balb/c mice treated with SAR-20351 (50mg/kg PO bid)

SAR-20351 was well tolerated with average bodyweights increasing steadily during the course of treatment. Complete blood counts showed no differences between vehicle and compound treated animals. On day 21 the T/C value was 34%.

FACS analysis of tissue from A20 tumor and B-cells from TDLN following treatment with SAR-20351

Digested and stained samples were run through an Attune NxT flow cytometer and samples analysed using Flowjo software. During FACS analysis PD response was assessed by measuring the MFI of several key proteins within tumor cells. Note: B-cells from tumor draining lymph nodes are assumed to be tumor cells but this cannot be verified.



Conclusions and Future Directions:

- SAR-20351 is a potent, selective, ATP-competitive inhibitor of TYK2 and reduces STAT phosphorylation downstream of cytokine stimulation in human cells and human and mouse whole blood
- In mouse syngeneic models of pancreatic, colorectal and kidney cancers, melanoma and B-cell lymphoma, SAR-20351 is well tolerated and inhibits tumor growth when dosed orally as monotherapy
- SAR-20351 is well tolerated when combined with other therapeutic agents such as 5-FU, everolimus and aCTLA-4 antibodies, and leads to additive inhibition of tumor growth
- Treatment with SAR-20351 affects cell populations in the tumor microenvironment with reductions in Tregs and MDSCs, and increases in CD8+ cells observed. Changes in expression levels of proteins such as PD-1, PD-L1 and Bcl-2 are observed on tumor and immune cells
- Compounds will be assessed in further syngeneic models as monotherapy and in combination with additional chemotherapeutics and antibodies
- SAR-20351 has been selected as a candidate for formal preclinical development

References:
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