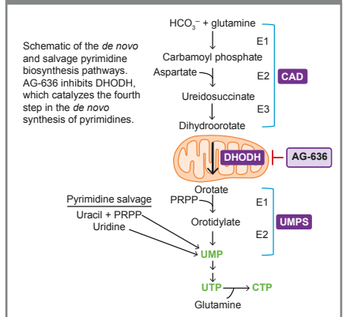
Hematologic malignancies exhibit selective vulnerability to inhibition of *de novo* pyrimidine biosynthesis by AG-636, a novel inhibitor of dihydroorotate dehydrogenase in phase 1 clinical trials

Danielle Ulanet[1], Victor Chubukov[1], John Coco[1], Gabrielle McDonald[1], Mya Steadman[1], Rohini Narayanaswamy[1], Sebastien Ronseaux[1], Sung Choe[1], Tabea Erdmann[2], Kevin Truskowski[1], Kavitha Nellore[3], Siva Sanjeeva Rao[3], Hosahalli Subramanya[3], Georg Lenz[2], Michael Cooper[1], Josh Murtie[1], Kevin Marks[1]

[1]Agios Pharmaceuticals, Inc., Cambridge, MA, USA; [2]Universitätsklinikum Münster, Münster, Germany; [3]Aurigene Discovery Technologies Ltd., Bangalore, India

BACKGROUND

Figure 1. Pyrimidine biosynthesis

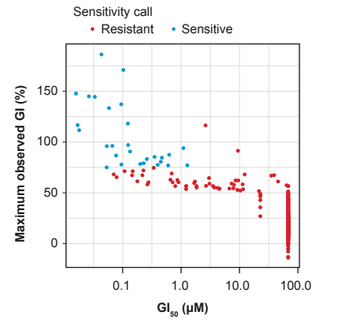


Pyrimidine synthesis enzymes: CAD, DHODH, UMPS
CAD = carbamoyl-phosphate synthetase 2 (E1), aspartate transcarbamylase (E2), and dihydroorotase (E3); CTP = cytosine triphosphate; PRPP = 5-phosphoribosyl-alpha-pyrophosphate; UMP = uridine monophosphate; UMPS = uridine monophosphate synthetase (orotate phosphoribosyl transferase [E1], orotidine-5'-decarboxylase [E2]); UTP = uridine triphosphate

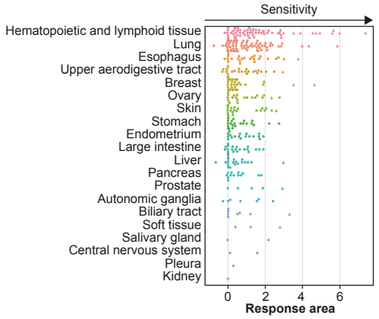
- Rapidly proliferating cells reprogram cellular metabolism to support increased biosynthetic demands, a feature that can expose targetable vulnerabilities for therapeutic intervention.
- We performed a chemical biology screen to identify metabolic vulnerabilities in particular tumor subtypes and identified that a novel inhibitor of dihydroorotate dehydrogenase (DHODH), AG-636, exhibited potent and selective growth inhibitory activity in cancer cell lines of hematologic origin. Cell lines of solid tumor origin exhibited comparatively poor sensitivity to AG-636.
- DHODH catalyzes the fourth step in the *de novo* synthesis of pyrimidines (**Figure 1**). Pyrimidines can also be made via salvage pathways; however, salvage pathways may be insufficient to satisfy pyrimidine demand in certain contexts.
- DHODH inhibition was pursued as an anticancer strategy in the 1990s using brequinar[1]; however, clinical development was discontinued owing to lack of efficacy in phase 1 studies encompassing diverse solid tumor types.[2] Patients with hematologic malignancies were not evaluated in these studies.
- We utilized AG-636 to further probe the basis for selective dependence on DHODH in malignant cells of hematologic versus solid tumor origin, both *in vitro* and *in vivo*.

RESULTS

Figure 2. Cell line panel screen identifies selective sensitivity of cell lines of hematologic origin to inhibition of DHODH



Activity of AG-636 in a panel of 395 cancer cell lines. Blue dots represent cell lines scored as sensitive (GI value of ≥75% and GI_{50} <1.5 μM).

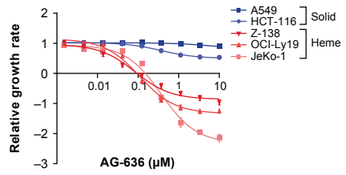


Sensitivity to AG-636 as a function of cell line lineage. Triangles represent sensitive lines.

Table 1. Sensitivity breakdown comparing cell lines of hematopoietic and lymphoid lineage versus all other cell lines screened

	Cell line lineage	
	Hematopoietic and lymphoid	All other
Number of sensitive cell lines (%)	11 (19)[a]	17 (5)
Number of resistant cell lines	47	320
Total	58	337

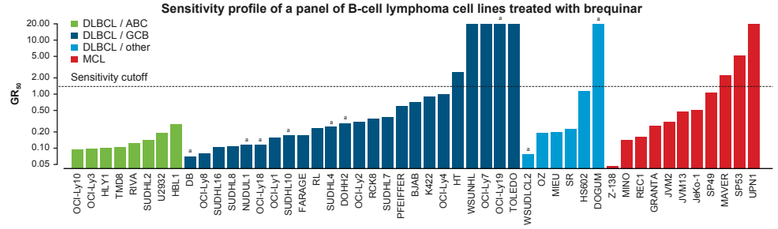
[a]p<0.001



In vitro sensitivity of the indicated cell lines to AG-636. Cells were treated for 96 hr. Growth rates are depicted relative to DMSO control.

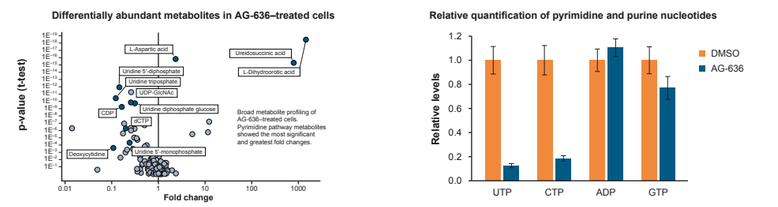
Response area = area under the GI versus drug concentration curve; DMSO = dimethyl sulfoxide; GI = growth inhibition; GI_{50} = half-maximal growth inhibitory concentration; heme = hematologic origin; solid = solid tumor origin

Figure 3. Broad responsiveness to DHODH inhibition across B-cell lymphoma subtypes



Cells were treated with brequinar in a 96-hr growth assay and cell number was assessed by CellTiter-Glo
[a]Cell lines reported as double-hit lymphoma
ABC = activated B cell; DLBCL = diffuse large B-cell lymphoma; GCB = germinal center B cell; GR_{50} = concentration of drug that resulted in a 50% reduction in growth rate; MCL = mantle cell lymphoma

Figure 4. AG-636 modulates cell metabolism and inhibits growth via on-target mechanisms



A panel of six cell lines was treated ± 1 μM AG-636 for 20 hr and metabolites were extracted. Left, broad metabolite profiling; Right, relative quantification of select nucleotides. Mean values ± SEM are depicted.

MV-4-11 cells were treated with 1 μM AG-636 or DMSO control for 20 hr in the absence or presence of the indicated concentrations of uridine. Pool sizes of UDP-glucose and dihydroorotate are indicated. Mean values ± SD are depicted.

MV-4-11 cells were cultured with increasing concentrations of AG-636 or DMSO control for 96 hr in the absence or presence of the indicated concentrations of uridine. Growth was measured using CellTiter-Glo. Growth rates are depicted relative to DMSO control.

ADP = adenine diphosphate; CDP = cytidine diphosphate; dCTP = deoxy-cytosine triphosphate; GTP = guanosine triphosphate; SEM = standard error of the mean; UDP = uridine diphosphate

Figure 5. Correlation between uridine depletion and effects on cell growth using real-time imaging

In the setting of depleted extracellular uridine, AG-636 results in immediate effects on viability of lymphoma cells, whereas solid tumor lines can maintain growth

Cells were cultured in the presence of 10 μM AG-636 or DMSO control with cell confluence measured every 2 hr. Media samples from replicate plates were collected at the indicated timepoints for uridine measurements. The figure shows the fold change in cell confluence relative to the 0 hr timepoint for each cell line (left axis) and absolute media uridine concentration (right axis).

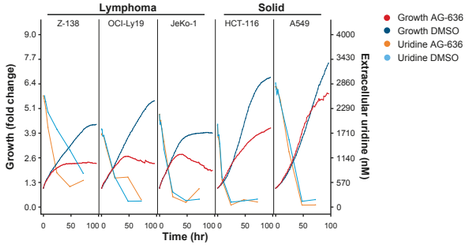
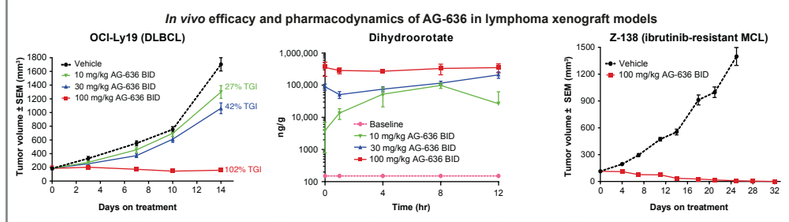


Figure 6. Strong *in vivo* antitumor activity of AG-636 in lymphoma models compared with poor activity in solid tumor models

***In vivo* efficacy and pharmacodynamics of AG-636 in lymphoma xenograft models**

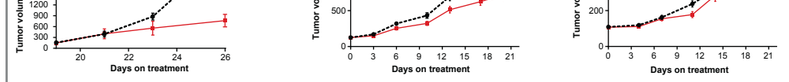


Efficacy. When tumors on OCI-Ly19 tumor-bearing mice reached an average size of ~200 mm^3, mice were treated with vehicle or AG-636 at a dose of 10, 30, or 100 mg/kg BID (n=15/group).

Dihydroorotate levels. Tumors were harvested at end of study and at the indicated timepoints following the last dose. Mean dihydroorotate concentrations ± SD are shown. Dihydroorotate levels in vehicle-treated tumors were below the quantifiable limit (pink dotted line).

Z-138 tumor-bearing mice were treated with vehicle or AG-636 at a dose of 100 mg/kg BID (n=15/group) for the indicated number of days. Complete tumor regression was observed in all mice in the AG-636–treated group.

***In vivo* efficacy of AG-636 in solid tumor xenograft models**



Ly11212 tumor-bearing mice were treated with vehicle or AG-636 at a dose of 100 mg/kg BID (n=3/group) for the indicated number of days.

HCT-116 or A549 tumor-bearing mice were treated with vehicle or AG-636 at a dose of 100 mg/kg BID (n=15/group) for the indicated number of days. Mean tumor volumes ± SEM for all experimental groups are plotted.

BID = twice daily; PDX = patient-derived xenograft; TGI = tumor growth inhibition

Table 2. Changes in tumor uridine concentrations from *in vivo* efficacy studies

Tumor uridine concentrations did not decrease (A549) or decreased to a lesser degree (HCT-116) in solid tumor xenograft models than in lymphoma models.

	Z-138	OCI-Ly19	HCT-116	A549
TGI, %	200	102	40	27
Tumor AG-636 exposure, $AUC_{0\text{-}12hr}$ hr·ng/g	18,600	22,000	49,700	56,000
Percentage decrease in tumor uridine	44	39	19	–3
	Lymphoma		Solid	

$AUC_{0\text{-}12hr}$ = area under the curve from 0 to 12 hr post dose

CONCLUSIONS

- AG-636 is a novel and potent inhibitor of DHODH that selectively impairs growth and viability of cancer cells of hematologic origin, with broad activity across lymphoma subtypes.
- Lymphoma and solid tumor cell lines exhibited a divergent ability to survive and sustain growth in the context of depleted extracellular uridine and DHODH inhibition, suggestive of adaptive mechanisms to supply pyrimidine pools and/or to cope with nucleotide stress in solid tumor cell lines.
- The lineage-selective reliance on DHODH translates to the *in vivo* setting, with AG-636 exhibiting strong antitumor activity across lymphoma models, including a highly aggressive triple-hit lymphoma model and an ibrutinib-resistant mantle cell lymphoma model.
- These studies support the development of AG-636 for the treatment of lymphoma. A phase 1 study has been initiated in patients with relapsed/refractory lymphoma (ClinicalTrials.gov NCT03834584).

Acknowledgments
The cell line panel screen was performed by Horizon Discovery, Cambridge, UK.
The lymphoma PDX model study was performed at EPO, Berlin-Buch, Germany.

Disclosures
This study was funded by Agios Pharmaceuticals, Inc.
DU, VC, JC, GM, MS, RN, SR, SC, KT, MC, JM, KM: Agios – employment and equity ownership. TE: no conflict of interest to disclose. KN, HS: Aurigene Discovery Technologies Ltd. – employment and equity ownership. SSR: Firmus Laboratories – employment and equity ownership. GL: Bayer, Celgene, Gilead, Janssen, Roche – consultant, honoraria, research funding, speakers bureau member; Agios – research funding; AstraZeneca – consultant, honoraria, research funding; Bristol Meyers Squibb – consultant.
Editorial assistance was provided by Shirley Louise-May, PhD, CMPP, Excel Medical Affairs, Fairfield, CT, USA, and supported by Agios.

References
1. Ullrich A et al. *Eur J Biochem* 2001;268:1861-8. **2.** Munier-Lehmann H et al. *J Med Chem* 2013;56:3148-67.

Scan code to receive PDF file of the poster or visit http://bit.ly/32bhsXb