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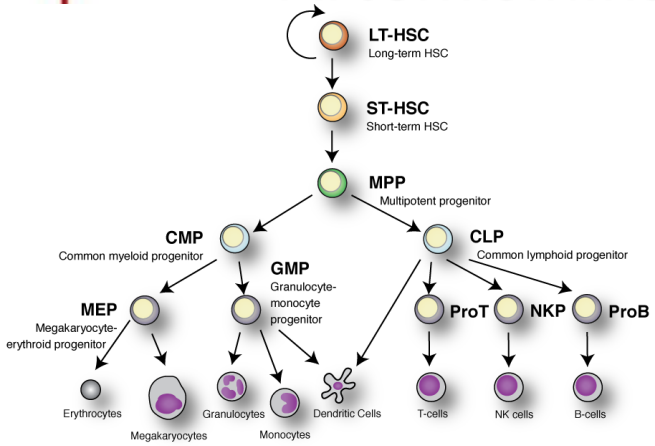
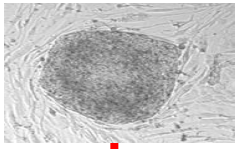
Artemisinins for Treatment of Acute Leukemias

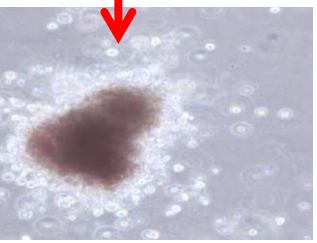


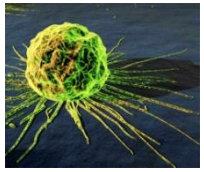
Curt Civin, MD

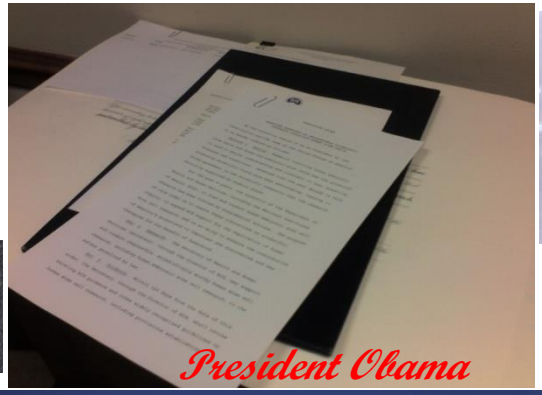
National LLS Research Update

April 13, 2016



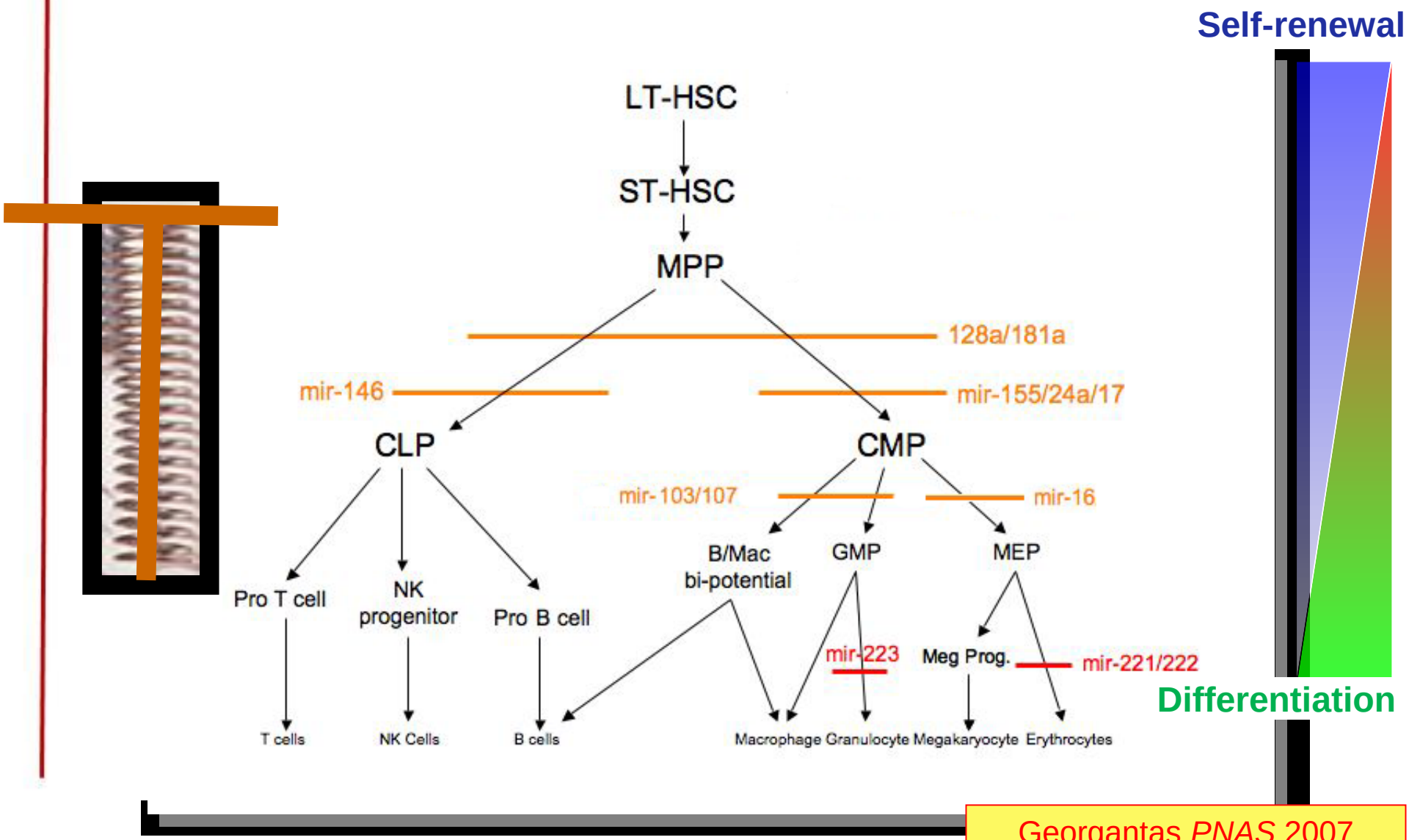






Bench ⟷ Bedside

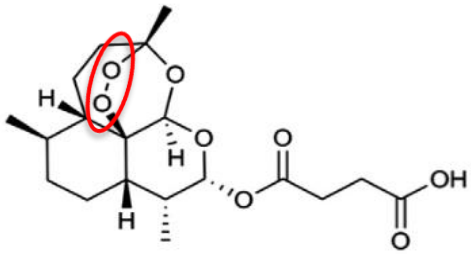
Informatic prediction that microRNAs might negatively regulate hematopoietic differentiation (coiled spring)



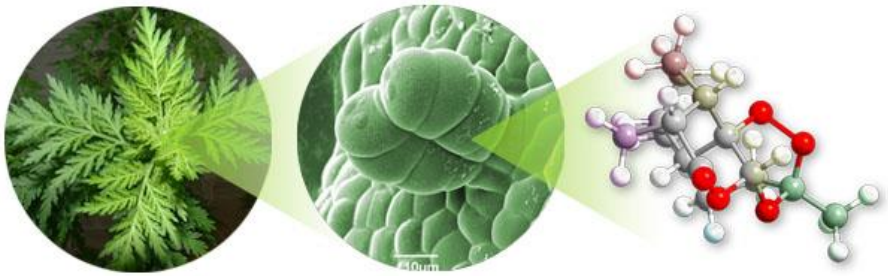
Georgantas *PNAS* 2007

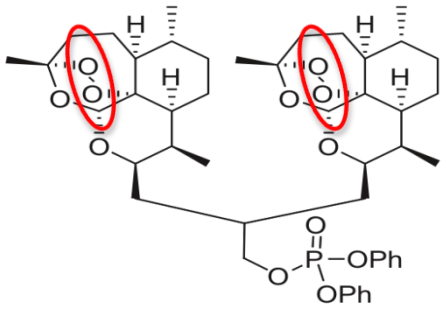
Repurposing Artemisinins for Acute Leukemia Treatment

- In a high-throughput screen of a clinical compound library, we identified that Artemisinins upregulate microRNA-34 and inhibit human leukemias
- Active in nearly every major cancer type *in vitro*
  - Of the NCI-60 panel, the most Artemisinin-sensitive cell lines were acute leukemias, but there are only 4 leukemias in that panel
- CEM (T-ALL) cell lines resistant to doxorubicin, hydroxyurea, methotrexate, or vincristine were not cross-resistant to Artemisinins
- Is there further preclinical basis to repurpose Artemisinins for leukemia treatment?
  - Artesunate (AS): in wide use for malaria treatment (2015 Nobel Prize to Youyou Tu); can do clinical trials of AS in leukemia now, we hope!
  - ART-838: a highly potent Artemisinin dimeric derivative; for the future?



Artesunate (AS)

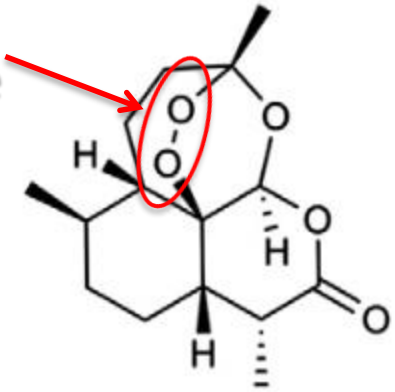




ART-838

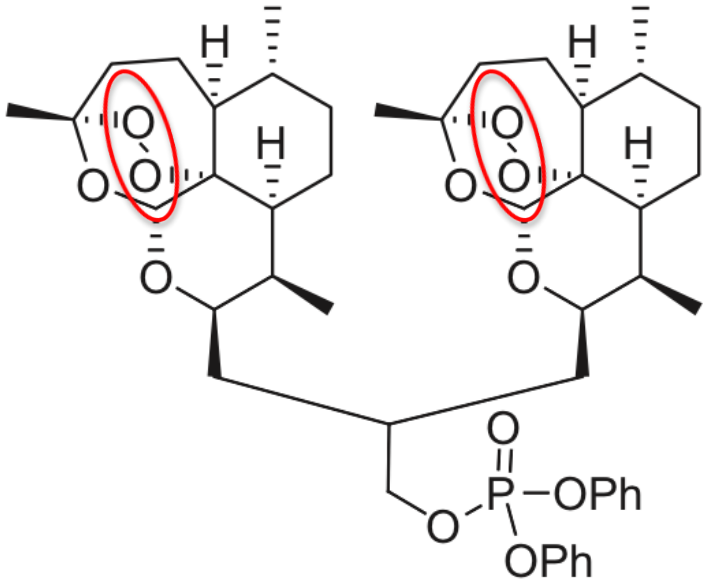
AS and ART-838

Endoperoxide pharmacophore

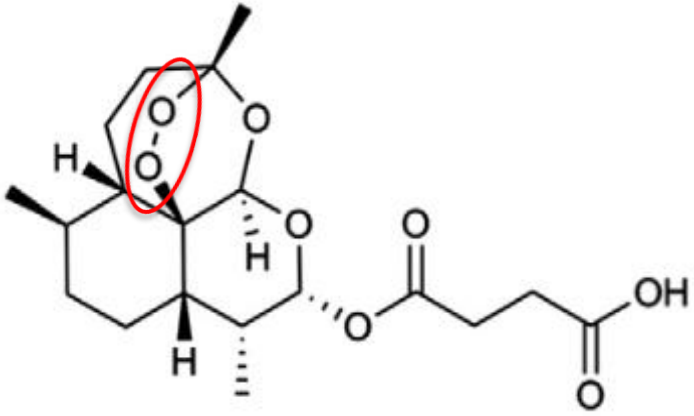


Artemisinin

Professor Gary Posner
JHU Dept of Chemistry



ART-838



Artesunate (AS)

Mott, *Bioorganic Chem* 2013

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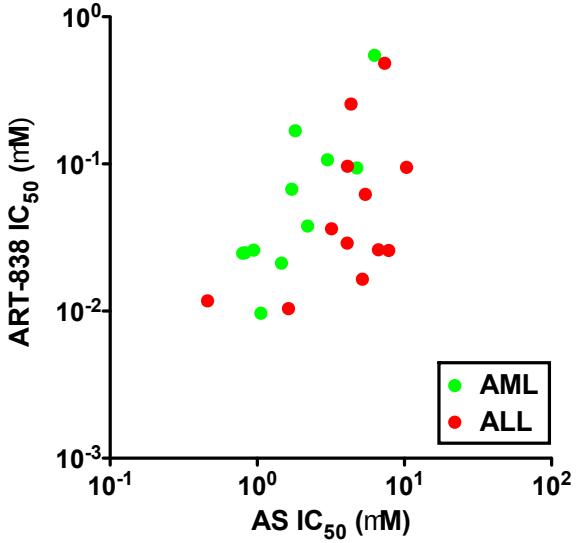
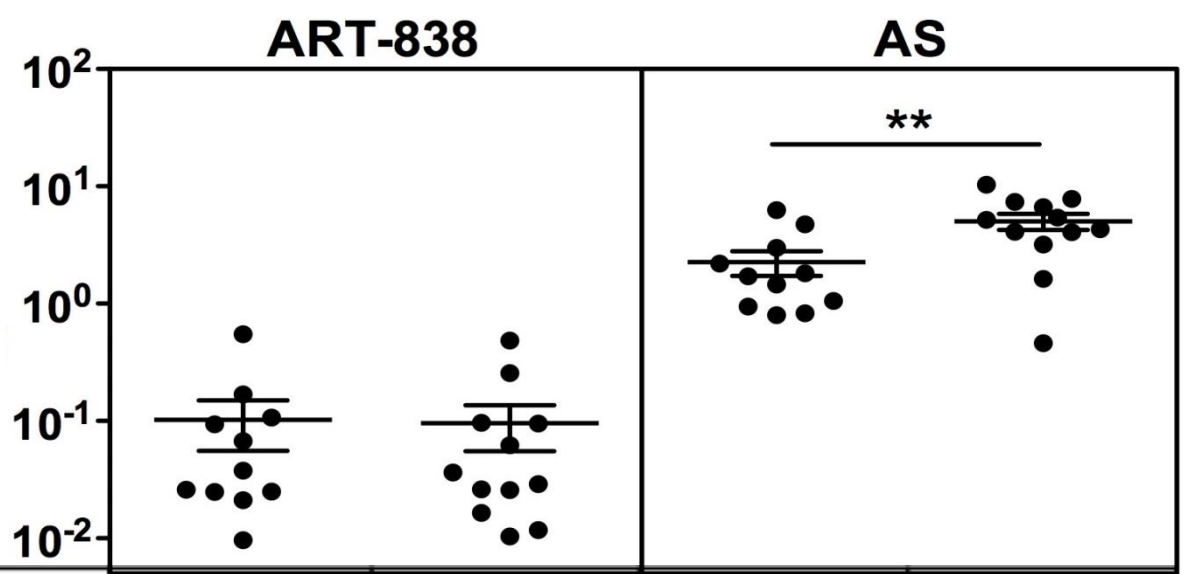
AS and ART-838 inhibited *in vitro* growth of *23/23* tested AML and ALL cell lines



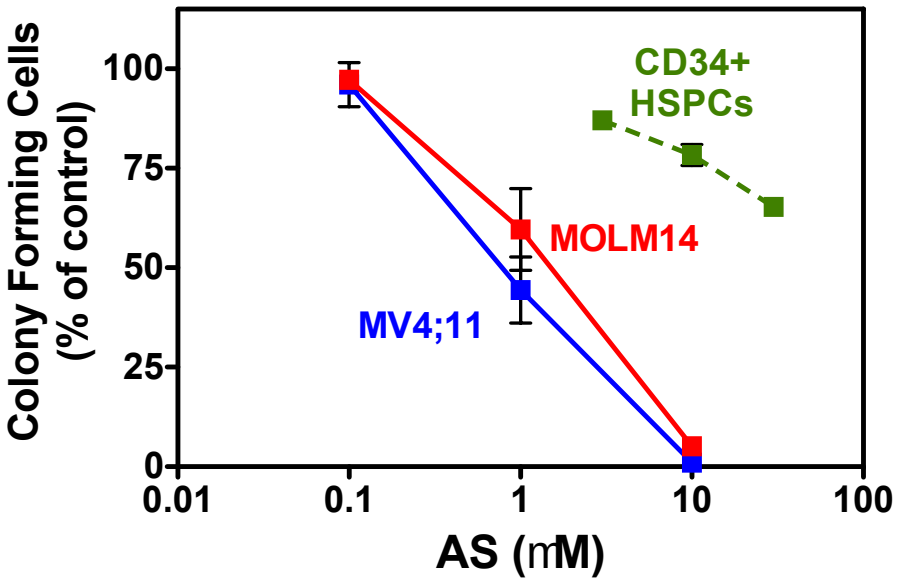


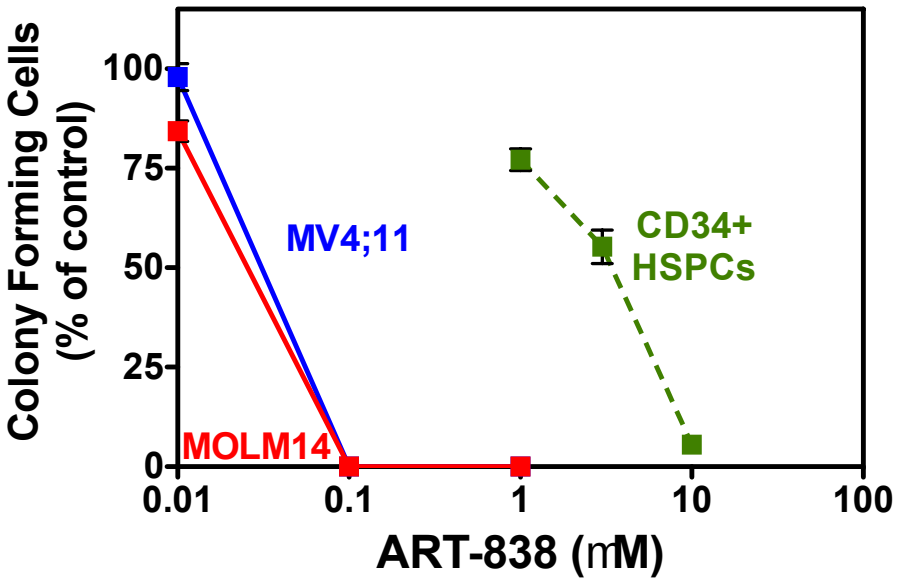
Leukemia Type	AML	ALL	AML	ALL
Mean IC_{50}	0.10	0.10	2.3	5.0
Median IC_{50}	0.04	0.03	1.7	4.8
IC_{50} Range	0.01-0.55	0.01-0.48	0.80-6.3	0.46-10
n	11	12	11	12

Fox, *Oncotarget* 2016

ART-838 is 88-fold (average) more potent than AS

AS and ART-838 were more cytotoxic to clonogenic leukemia cells than to normal hematopoietic colony-forming cells





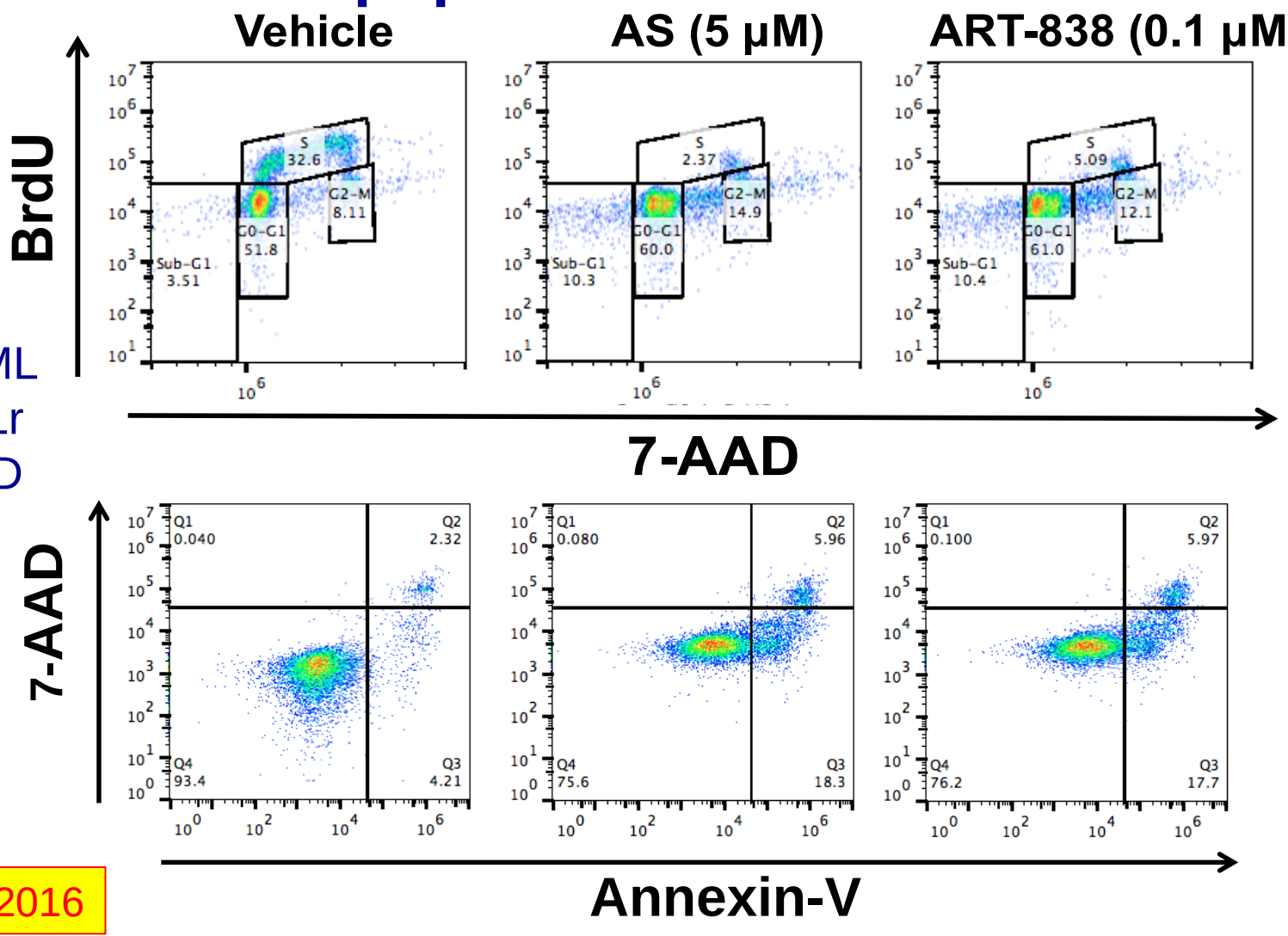
Suggests a therapeutic window

Similar results were obtained for HL60 and K562 cells

Fox, *Oncotarget* 2016

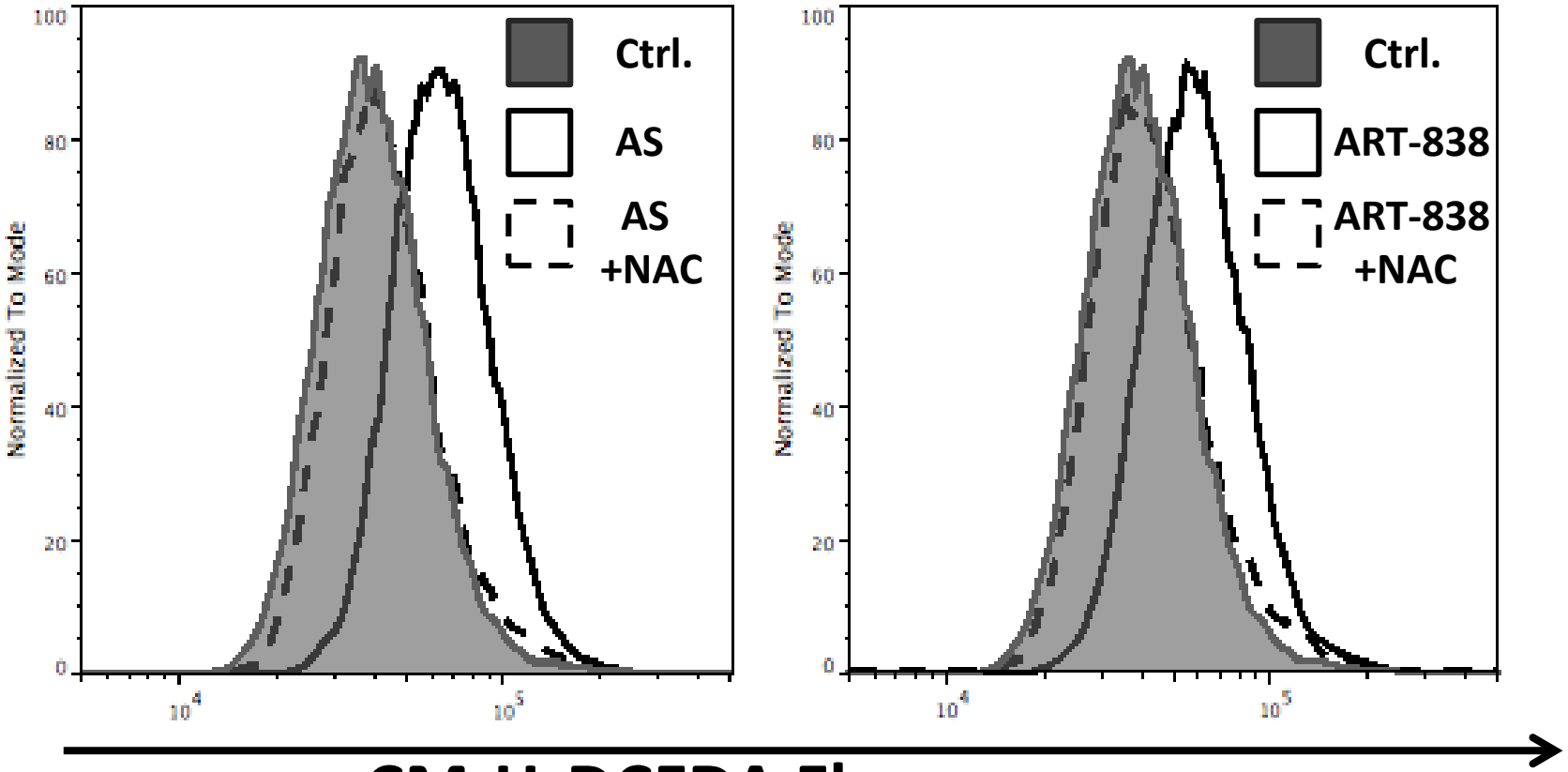
AS and ART-838 inhibited cell cycle progression and induced apoptosis in leukemia cells

MOLM14 AML cell line: MLLr and FLT3-ITD



Fox, *Oncotarget* 2016

AS and ART-838 induced generation of reactive oxygen species (ROS) that is inhibited by NAC



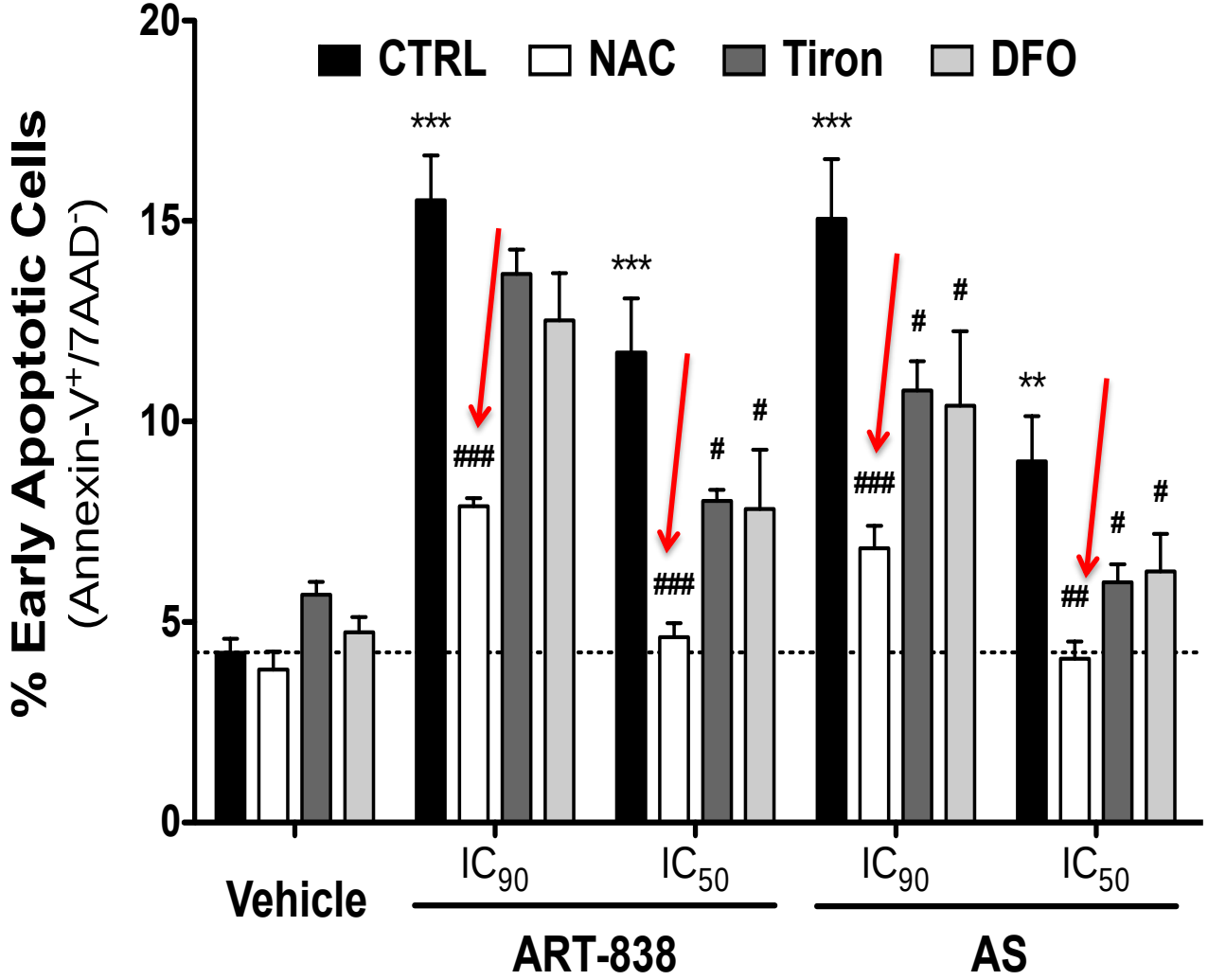
CM-H_2DCFDA Fluorescence

(ROS-sensitive dye)

NAC – intracellular glutathione precursor and ROS scavenger

Fox, *Oncotarget* 2016

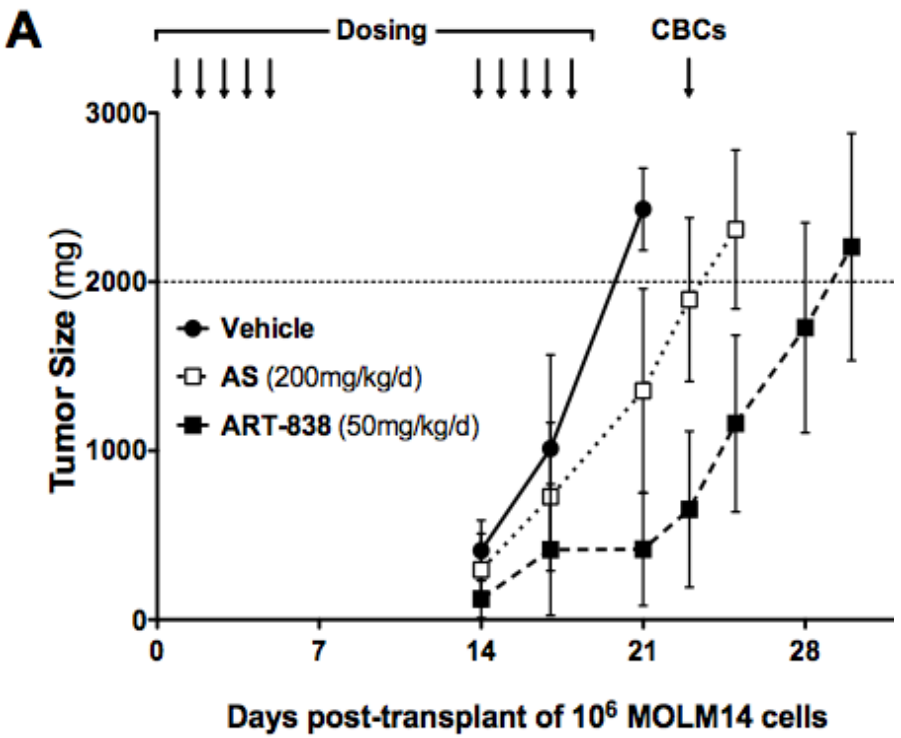
ROS inhibitors protected against AS- and ART-838-induced apoptosis



Fox, *Oncotarget* 2016

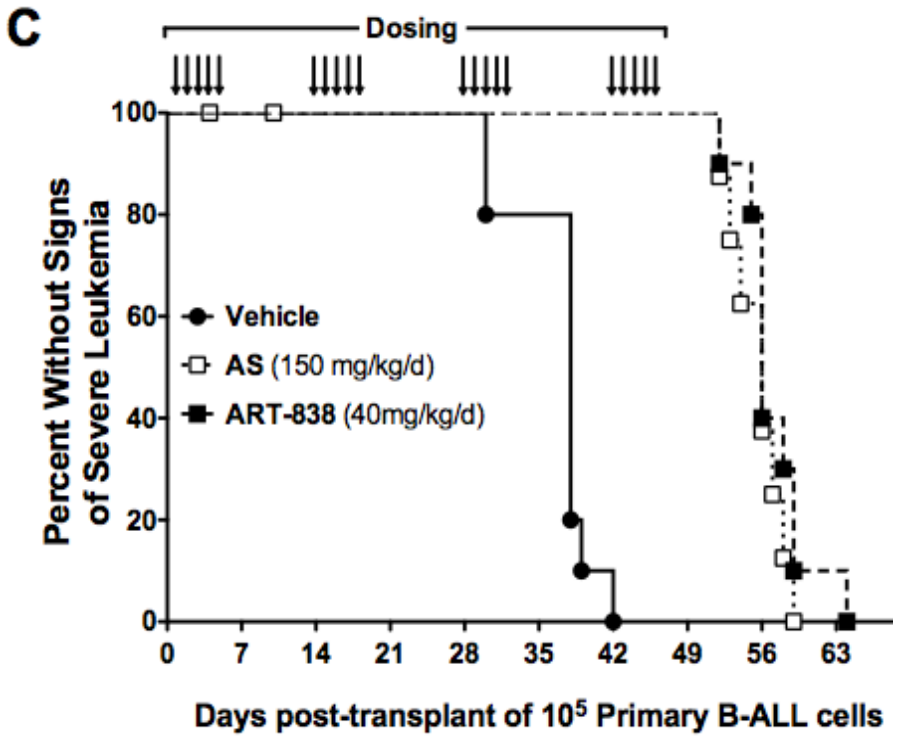
AS and ART-838 inhibited growth of human acute leukemias in NSG mice

Tumor growth: Xenograft



MOLM14 cell line sc xenograft model

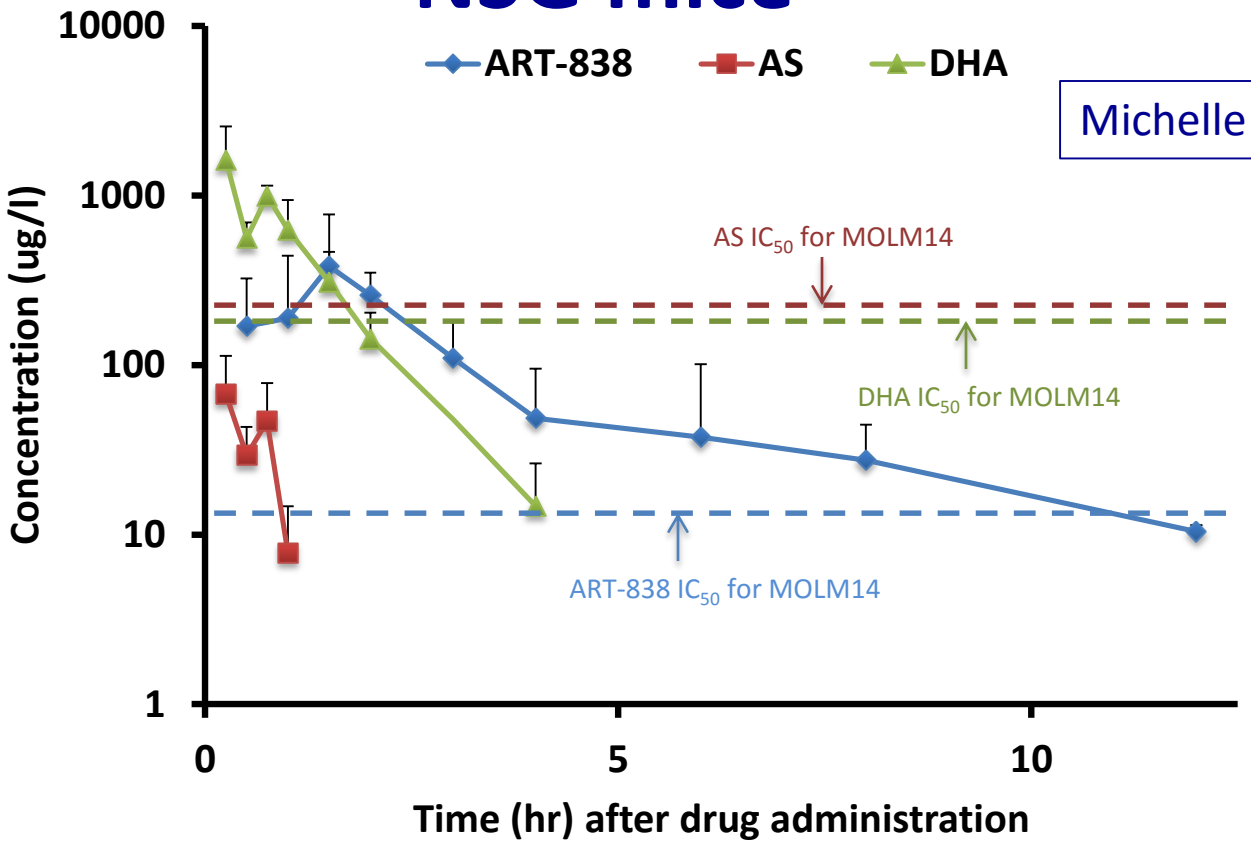
Survival: Primagraft



Primary ALL case #109 B-ALL primagraft model (from Pat Brown)

Fox, *Oncotarget* 2016

Pharmacokinetics of AS, DHA, and ART-838 in NSG mice



Michelle Rudek-Renaut

Projected ART-838 efficacy against acute leukemias for ~6h after an oral dose

AS, DHA (major metabolite of AS) and ART-838 plasma concentrations in NSG mice treated with a single dose of AS (200 mg/kg) or ART-838 (50mg/kg). Symbols represent concentrations of compound (3-6 mice/time point) by high performance liquid chromatography with tandem mass spectrometric detection. Dotted lines represent *in vitro* IC_{50} of ART-838, AS or DHA for MOLM14 cells.

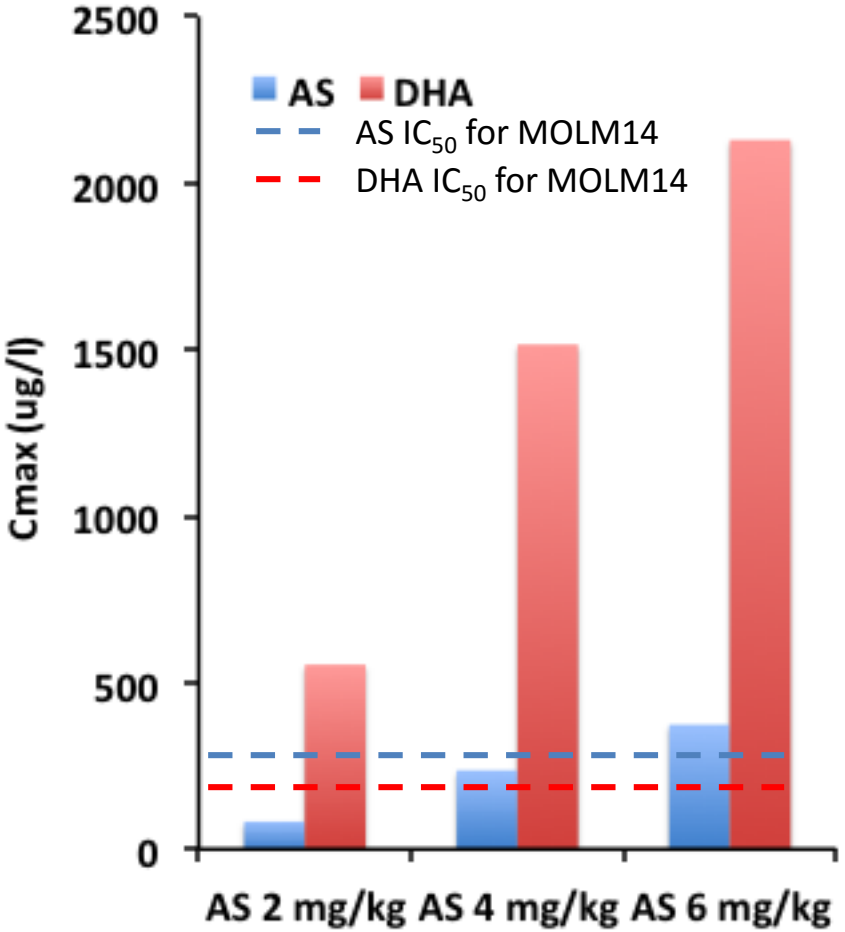
Fox, *Oncotarget* 2016

Pharmacokinetics and Pharmacodynamics of Oral Artesunate Monotherapy in Patients with Uncomplicated *Plasmodium falciparum* Malaria in Western Cambodia

David Saunders,[a] Phisit Khemawoot,[a,h] Pattaraporn Vanachayangkul,[a] Raweewan Siripokasupkul,[a] Delia Bethell,[a] Stuart Tyner,[a] Youry Se,[a] Wiriya Rutvisuttinunt,[a] Sabaithip Sriwichai,[a] Lon Chanthap,[a] Jessica Lin,[d] Ans Timmermans,[a] Doung Socheat,[b] Pascal Ringwald,[c] Harald Noedl,[a] Bryan Smith,[f] Mark Fukuda,[g] and Paktiya Teja-Isavadharm[a]

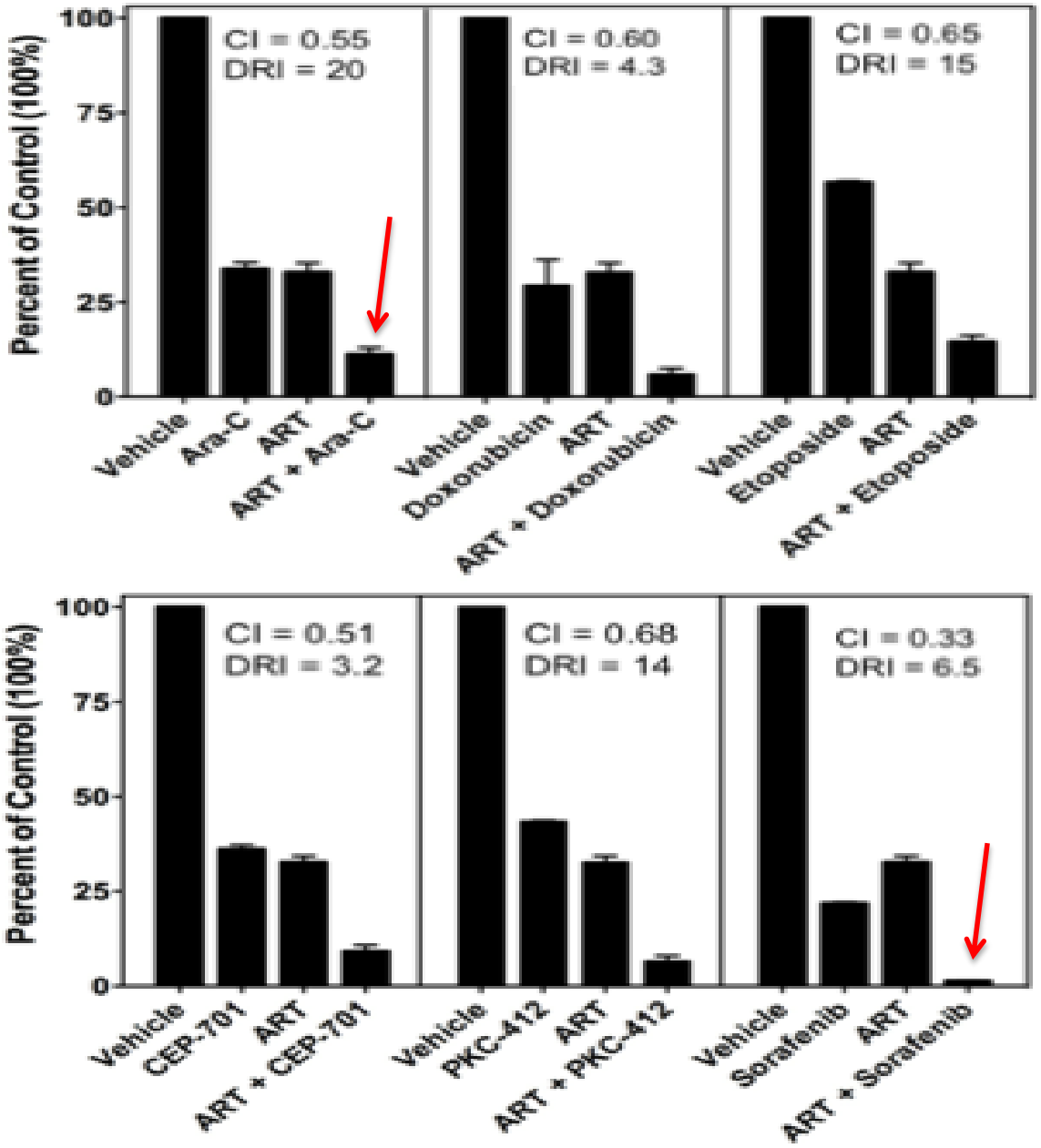
TABLE 1 Summary of overall pharmacokinetic parameters for subjects taking oral artesunate for 7 days

PK parameter	Geometric mean value for subjects given the following dose of AS:			*P* value[d]
	2 mg/kg	4 mg/kg	6 mg/kg	
AS on day 0[a]				
$t_{1/2}$ (h)	0.489	0.499	0.461	0.827
T_{max} (h)	0.482	0.518	0.640	0.366
C_{max} (μg/liter)	83.1	237	374	<0.0001
AUC_{0-8} (μg · h/liter)	91.2	279	453	<0.0001
MRT (h)	1.07	1.06	1.17	0.749
V/*F* (liter/kg)	14.2	10.1	8.56	0.051
CL/*F* (liter/h/kg)	20.1	13.9	12.9	<0.0001
DHA on day 0[b]				
$t_{1/2}$ (h)	0.838	0.913	1.13	0.170
T_{max} (h)	1.26	1.15	1.44	0.310
C_{max} (μg/liter)	554	1,515	2,129	<0.0001
AUC_{0-8} (μg · h/liter)	1,331	3,854	5,989	<0.0001
MRT (h)	2.06	2.21	2.84	0.008



Antimicrobial Agents and Chemotherapy 2012; 56: 5484–5493

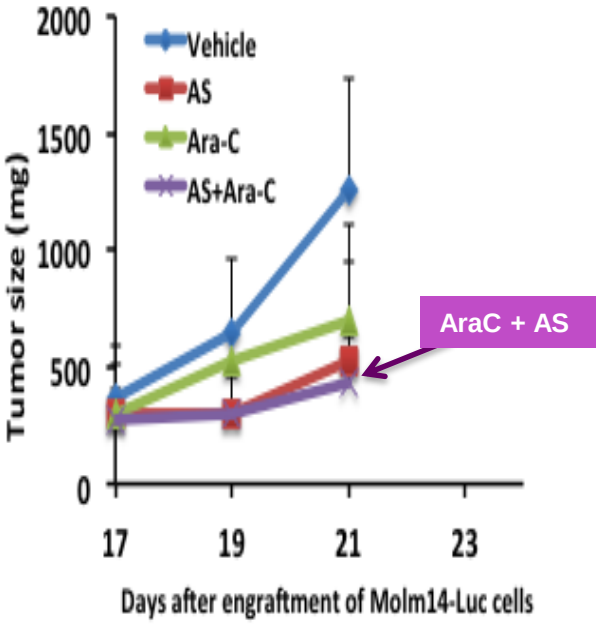
AS synergized with traditional antileukemics and tyrosine kinase inhibitors (KOPN8 cells)



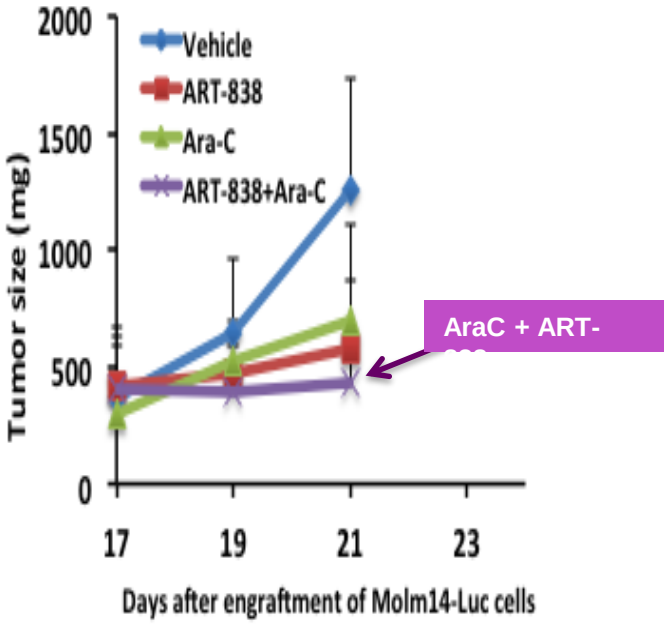
Fox, *Oncotarget* 2016

Either AS or ART-838 AS enhanced the *in vivo* efficacy of AraC against MOLM14 xenograft growth

AraC + AS

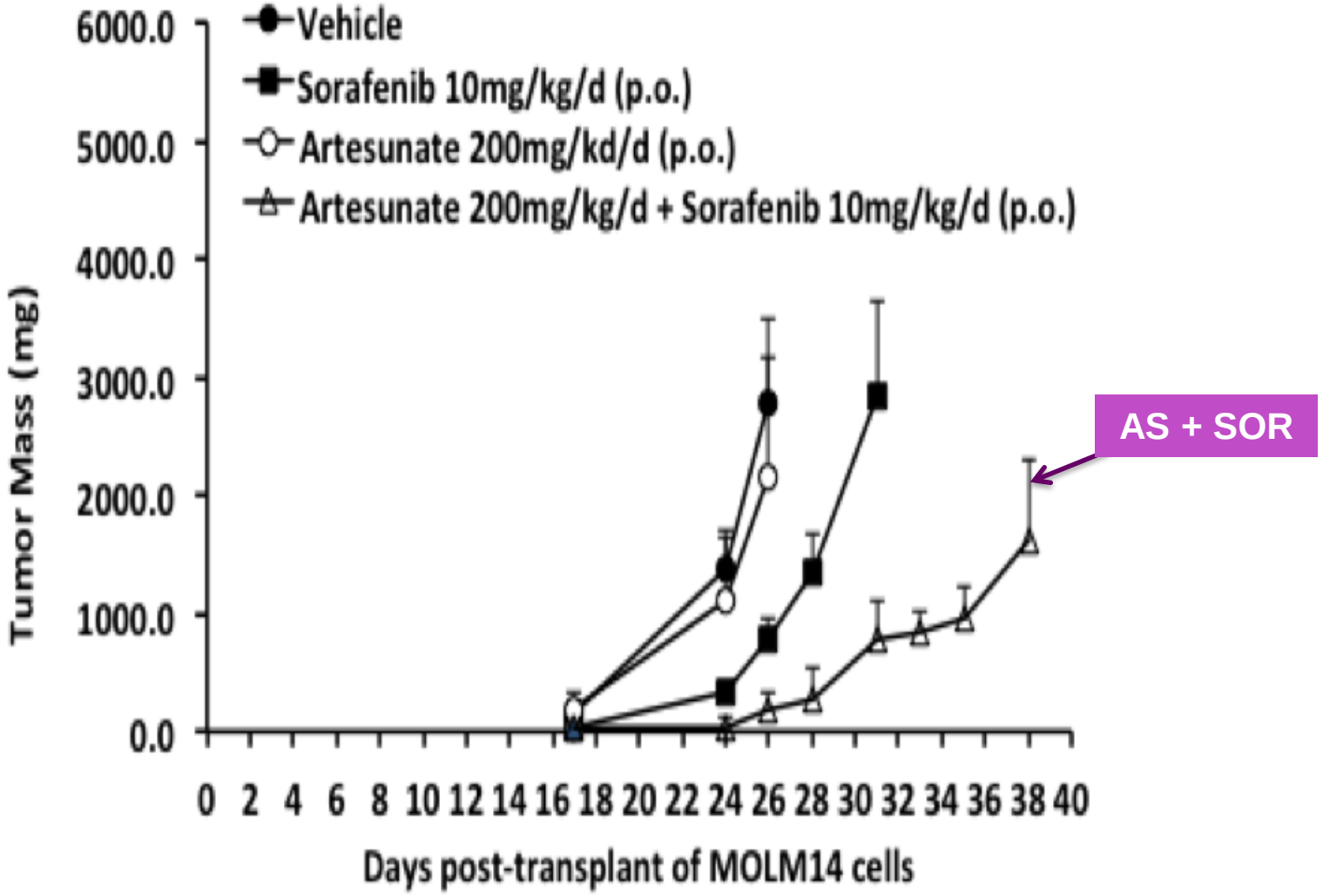


AraC + ART-838

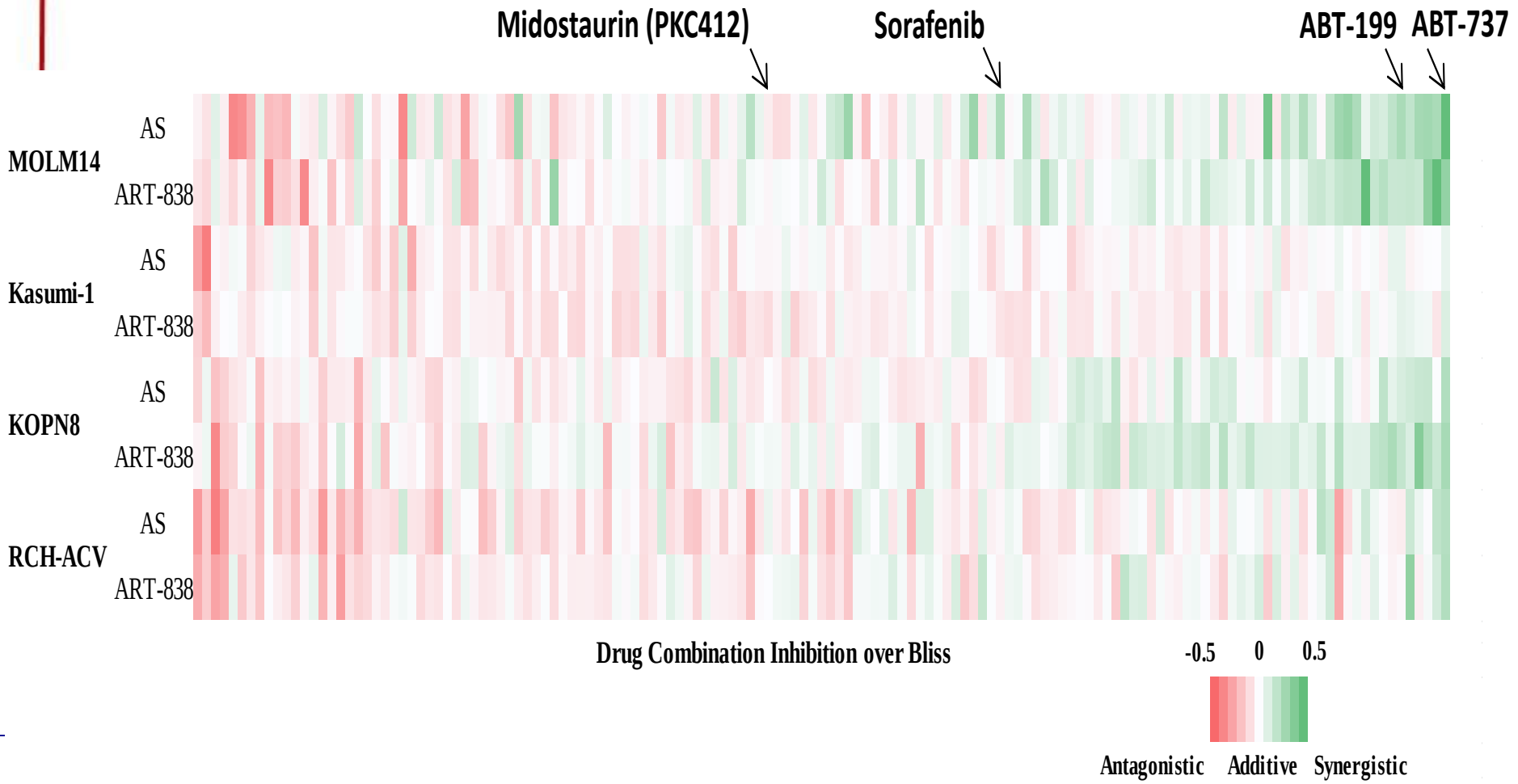


AraC: 40mg/kg/d IP qMTuWThF
AS: 250mg/kg PO qMWF
ART-838: 200mg/kg PO qMTh

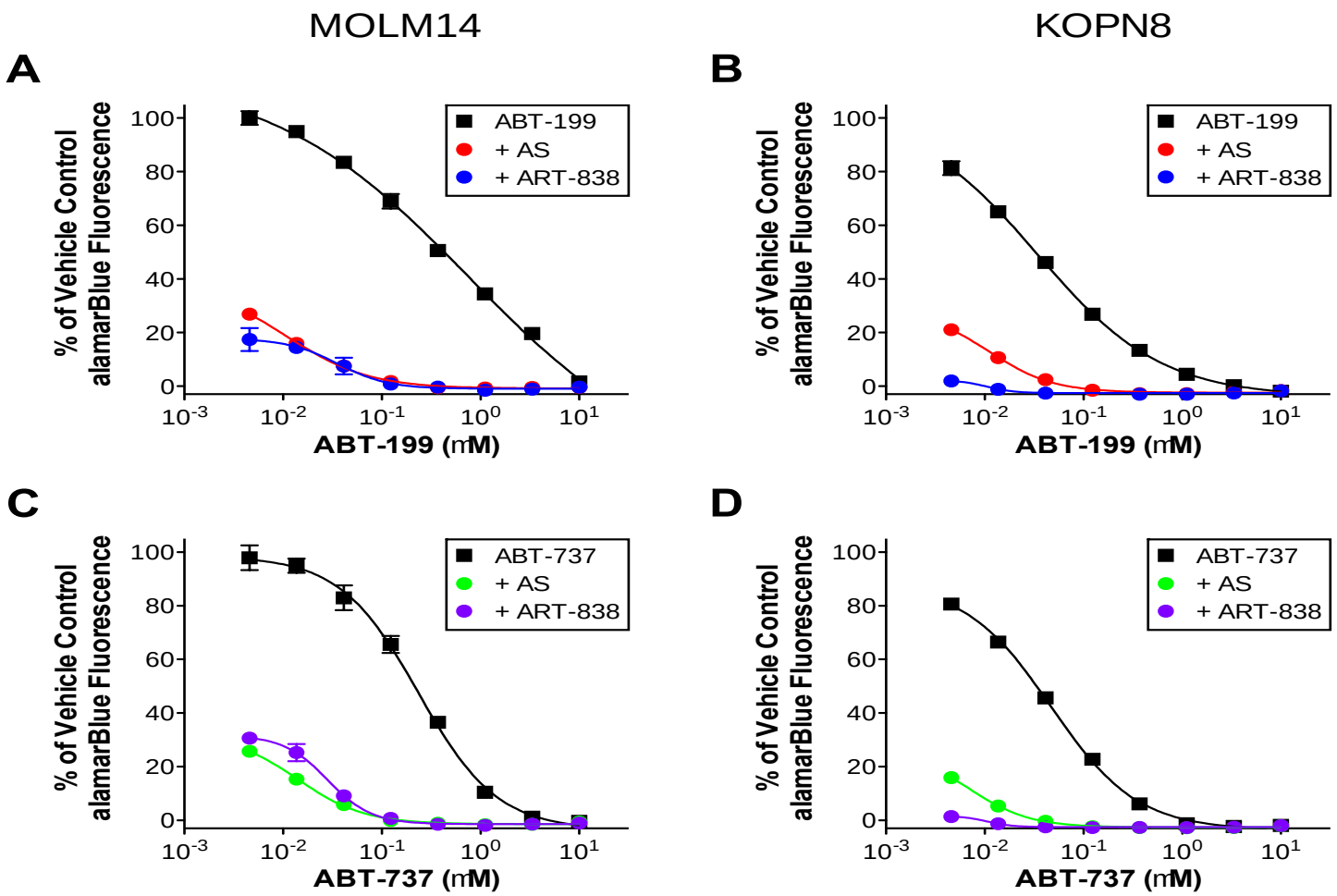
AS enhanced the *in vivo* efficacy of Sorafenib against MOLM14 xenograft growth



Synthetic lethal screen of 122 different drugs at 7 concentrations plus AS or ART-838 against 4 leukemia cell lines identified BCL2 inhibitors as the top hits

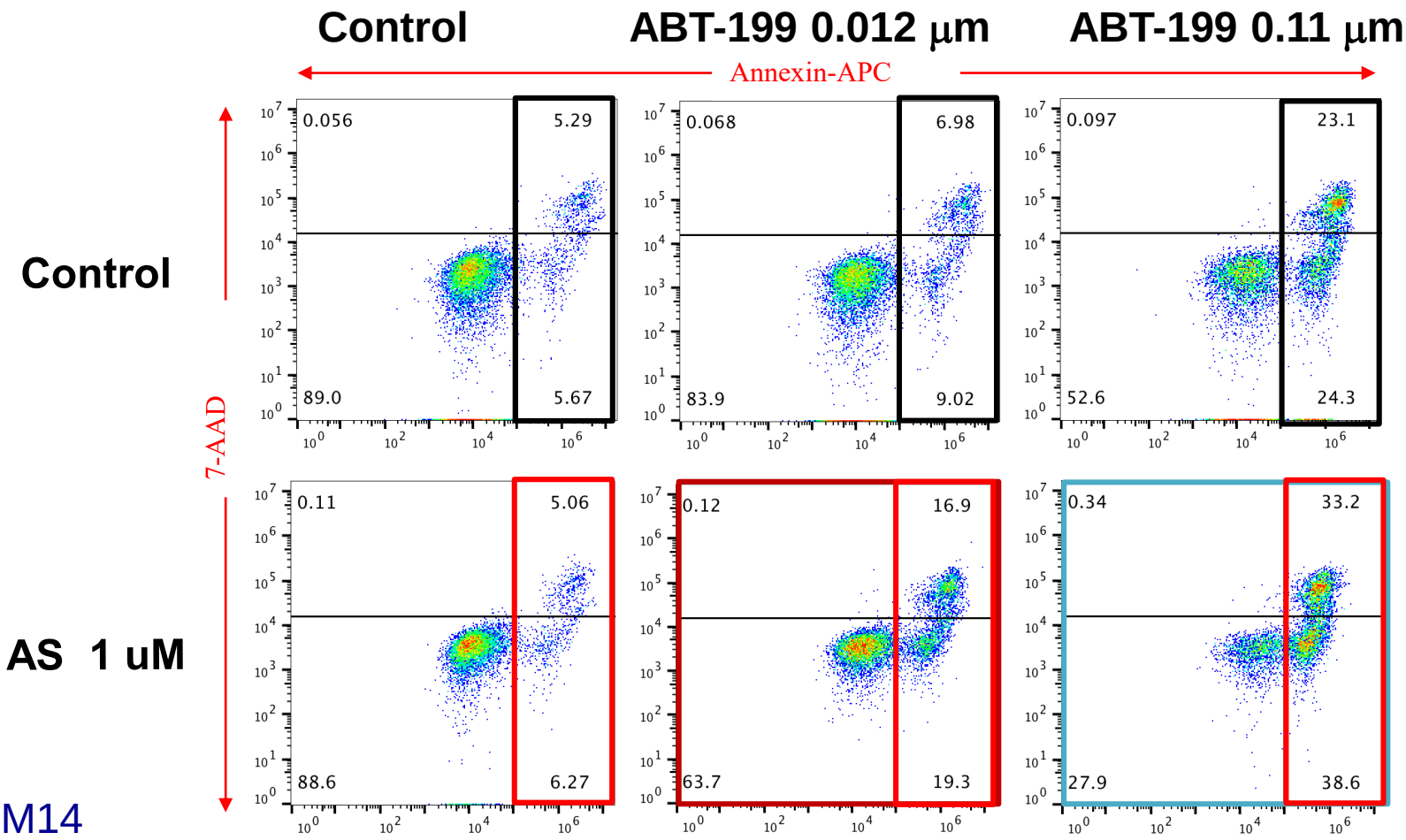


AS and ART-838 synergized with BCL2 inhibitors in validation assays



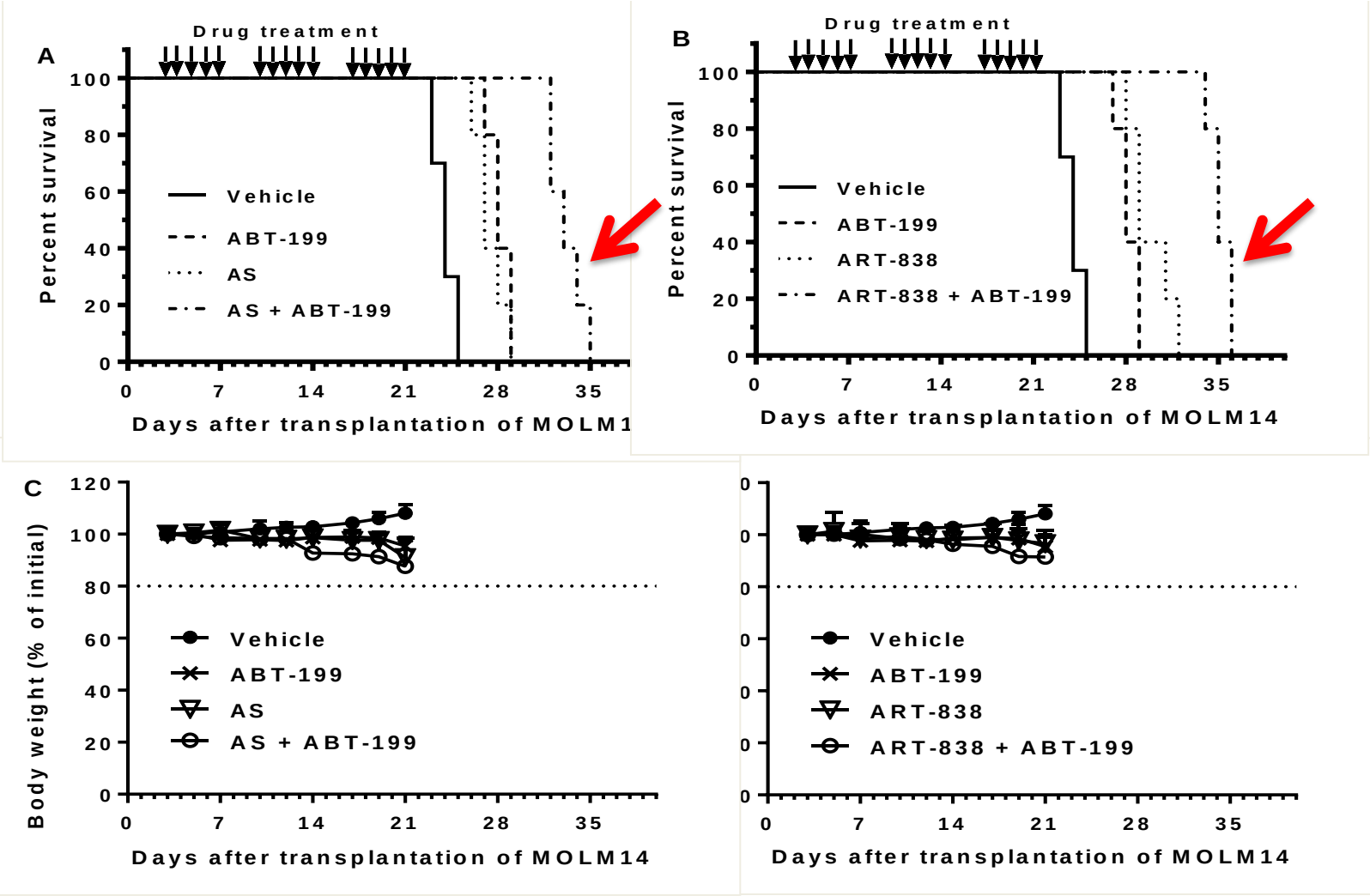
AS and ART-838 tested at their IC_{50}s

ABT-199 and AS cooperated to induce apoptosis



MOLM14

ABT-199 and either AS or ART-838 cooperated to inhibit MOLM14 growth *in vivo*



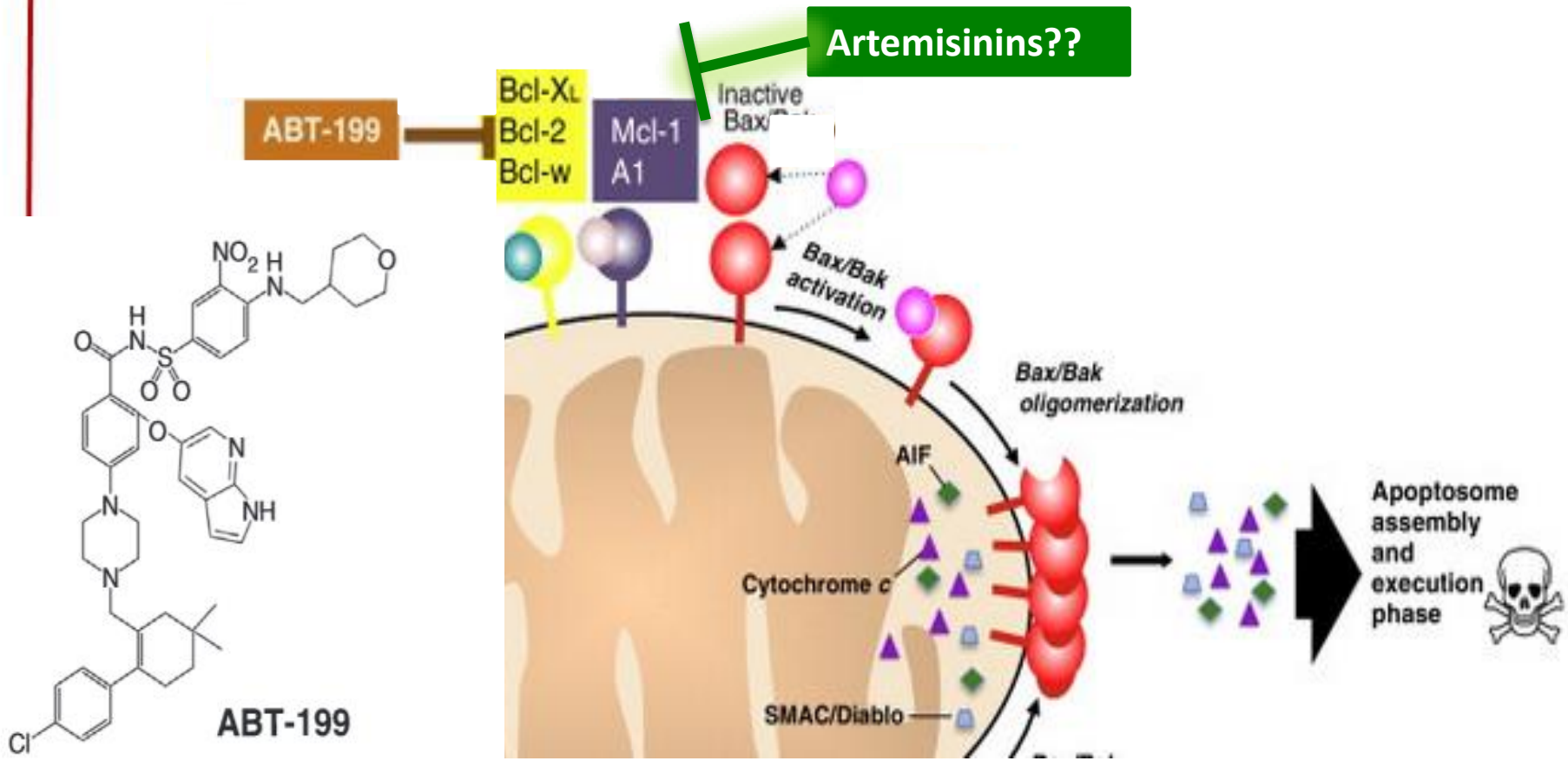
AS: 75 mg/kg/d PO qM-F

ART-838: 20 mg/kg/d PO qM-F

+ ABT-199: 100 mg/kg/d PO qM-F

What's the basis for the synergy?

Anti-apoptotic Bcl-2 family



Next Steps

- **Optimize dose-schedules of AS and ART-838 paired with ABT-199 in xenograft and primagraft models**
  - Efficacy
  - PK/PD
- **Probe synergistic antileukemic mechanisms of AS/ART-838 with ABT-199**
  - Determine expression of BCL-2 family members in AML cases
  - Investigate interplay between BCL-2 family members and AS or ART-838 in multiple AML cell lines
- **Clinical study: Conduct Phase I/II clinical trial of AS in acute leukemias w/ proof-of-mechanism PD**
  - PK/PD to integrate w/ xenograft expts and maximize exposure to AS to guide maximization of the future effects of the 2 synergistic drugs given together in patients

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Thanks!!

Xiaochun Chen PhD Asst Professor	Jennifer Fox PhD (2015)	Jamie Moynihan MS Research Tech	Kalyani Kumar UMSOM 2018
			

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