Targeting Integrin Signaling in Atherosclerotic Cardiovascular Disease (ASCVD)

John W. Craft Jr. [1,2], Amy Caivano[2], Anna Kazansky[2], Shahrzad Abbasi[2], Reiko Chamberlain, Qian Xiang, Anuj Kurella, Elizabeth Merlinsky, Xiao Li, Yi Zhao, Robert V. Market, Ron Biediger[2], Robert Market[2], Frederick Meece[2], Darren G. Woodside[2]

1) U Houston 2) Texas Heart Inst.

Background

In monocytes, integrin cell adhesion molecule signaling via the tyrosine kinase Syk is a very early event leading to expression of IL-1β and other pro-inflammatory cytokines. This occurs through the direct interaction between integrin β-chain cytoplasmic domains and the tandem SH2 domains of Syk. SYK (spleen tyrosine kinase) is a nonreceptor tyrosine kinase[1] originally associated with immune response receptor signaling, as SYK can be activated by binding to dually phosphorylated immunoreceptor tyrosine-based activation motifs (pITAMs). However, SYK is also activated upon integrin clustering. As Atherosclerosis has been shown to be an inflammatory disease, we describe here a drug discovery and development program targeting the integrin/Syk signaling axis, as a novel means to treat residual inflammatory risk in ASCVD.

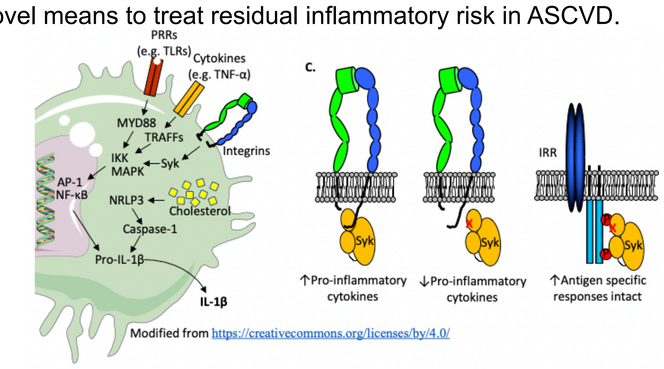


Figure 1: Integrin signaling via the non receptor tyrosine kinase Syk is an early event in monocyte/macrophage expression of a number of pro-inflammatory cytokines like IL-1β. Signaling is mediated through direct interaction of Syk with integrin cytoplasmic domains. Targeting this interaction with small molecules could lead to a novel approach to limit inflammation but keep other Syk functions, such as Fc and BCR signaling intact.

Methodology

A structure based computational screen of 15+ million compounds identified 1037 compounds clustered into five families based on their energy scores, functional groups, and Tanimoto structural similarity. Datamining this information using GAUSSIAN, AUTODOCK, GROMACS, and PYTHON tools through the University of Houston computing clusters and on AZURE cloud facilitated analysis of the compounds and identified candidates for synthesis and screening. Almost 200 compounds from this virtual screen that demonstrated stable Syk interactions in molecular dynamics simulations were synthesized and submitted for screening in cell-based integrin signaling assays, cell free integrin/SYK elisa's, thermal shift assays (TSAs), and cell-based FcγRI signaling selectivity assays. Three classes of compounds met our internal potency metric for identifying hits (~10 μM in the integrin signaling assay). Analogs of these classes have been designed and synthesized to develop structure activity relationships (SAR). To date, over 400 compounds have been synthesized and screened and we have identified 4 pharmacophore classes. Notably, we have improved potency in class 4 compounds by over 1000-fold.

For patent purposes, structures are not disclosed here.

Results

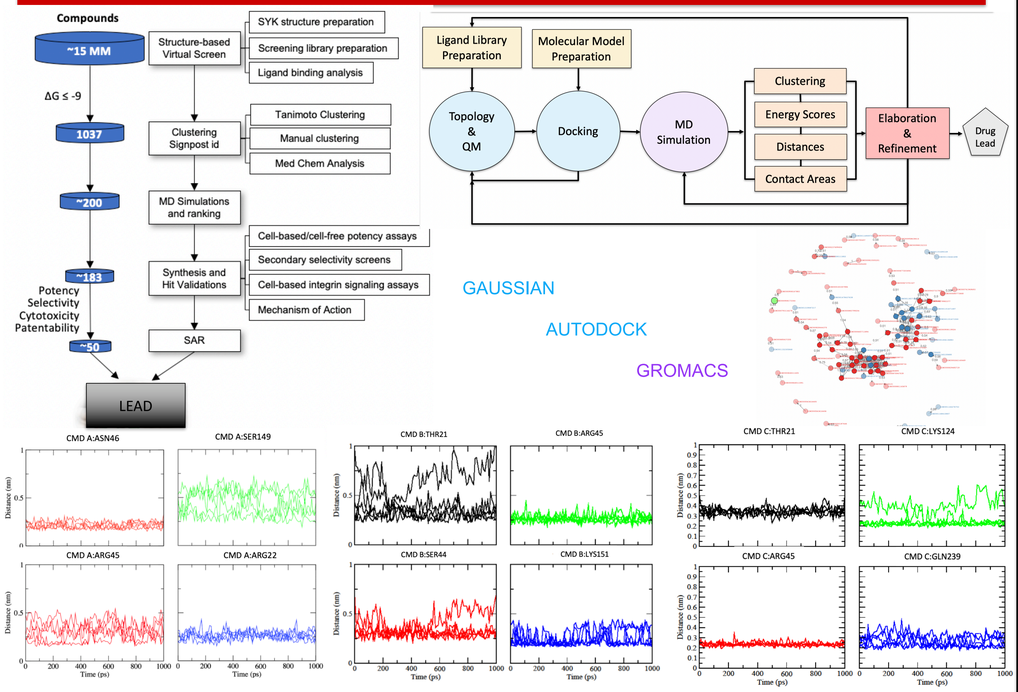
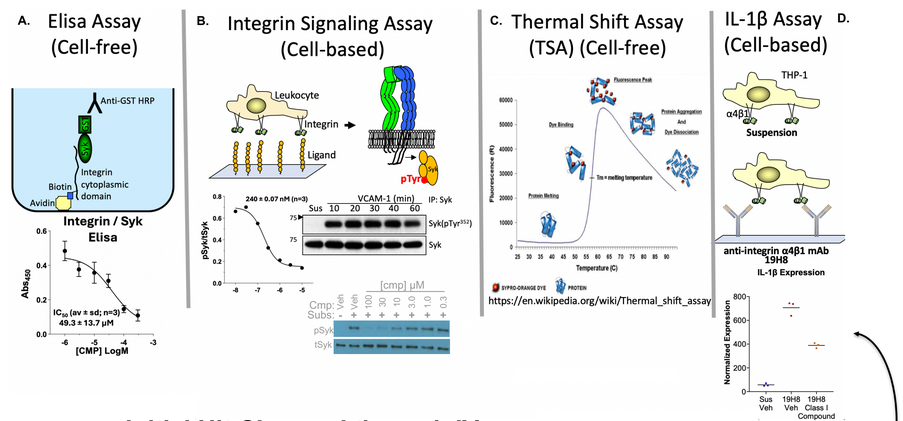


Figure 2: TOP) Our workflow includes an end-to-end process that starts with quantum mechanics (GAUSSIAN) to generate topologies and uses docking (AUTODOCK) as a rapid initial condition generator for interactors that can be further evaluated in a full simulation environment like GROMACS. This workflow is iterative at many levels, especially through the experience of our medicinal chemists. BOTTOM) MD 5 nano second simulations (CMD 1,2,3) indicate that SYK residues ARG22, ARG45, ASN46, THR21, LYS151, and GLN239 can form close associations with some of our lead ligands.



Initial Hit Classes I through IV

Class I	Class II	Class III	Class IV
Original Hit	Original Hit	Original Hit	Original Hit
SBVS G = -9.21	SBVS G = -7.784	SBVS G = -9.21	SBVS G = -8.738
tPSA = 99.7	tPSA = 74.48	tPSA = 99.7	tPSA = 57.5
cLogP = 2.71	cLogP = 3.45	cLogP = 2.71	cLogP = 5.57
Int Sig IC_{50}	Int Sig IC_{50}	Int Sig IC_{50}	Int Sig IC_{50}
1.6 ± 0.8 uM	5.1 ± 2.2 uM	9.6 ± 0.8 uM	6.8 ± 4.0 uM
TSA "Kd"	TSA "Kd"	TSA "Kd"	TSA "Kd"
11.7 uM	13.2 uM	13.7 uM	11.7 uM

Improving Potency in Class IV

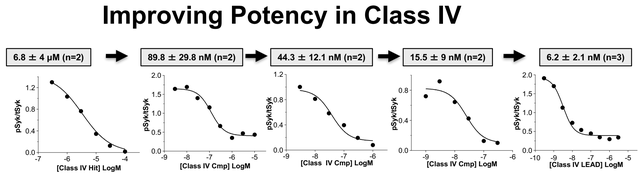


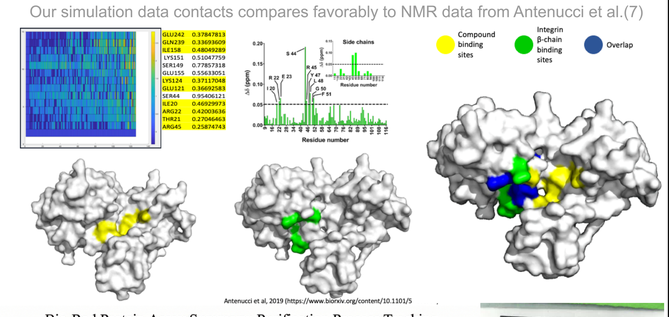
Figure 3: A) The integrin / SYK elisa for one of our a signpost compound derivative of Class I (compound A).
B) The integrin signaling assay from compound A. This family of compounds is expanding to include 33 variations to explore SAR; all of these derivatives go back into our computational process to further investigate putative interactions.
C) Thermal Shift Assay – compounds routinely cause a decrease in Tm Syk tandem SH2 domains indicating possible allosteric mechanism.
D) Signpost compound from Class I inhibition of integrin α4β1 dependent expression of IL-1β in THP-1 cells.

Figure 4: Above are a series of dose-response inhibition curves that evaluate integrin signaling IC_{50} values, the ligand concentration at which 50% of the phosphorylated signal is inhibited. Ligands B, C,and D evolved from the initial **SAR (Structure-Activity Relationship)** exploration of Class I and III comopunds. By strategically merging key pharmacophoric elements from earlier iterations, these ligands represent a 'best-of-both-worlds' approach—achieving low-nanomolar potency while maintaining the maximal signal suppression required to fully disrupt the integrin-Syk signaling axis.

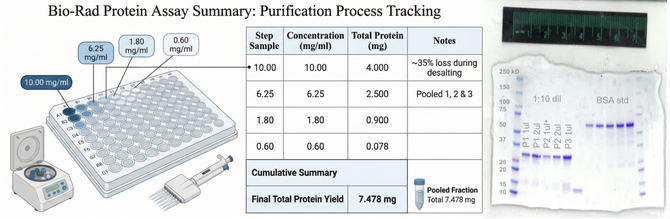
Conclusions

We have screened ~15M compounds virtually and **>400 "hit" compounds** have been synthesized. We have identified at least 4 distinct compounds scaffolds and improved potency in one class of compounds >1000 fold (lead compound IC_{50} = 6.7 nM). Compounds directly interact with Syk demonstrating the integin/Syk interaction is druggable. Our lead class has oral bioavailability (not shown) and is stable in Human Liver Microsome assays ($t_{1/2}$ = 157 min). We are testing PK in rodents to prepare for in vivo assessments in models of atherosclerosis, and preparing NMR experiments to determine mode of binding.

Our simulation data contacts compares favorably to NMR data from Antenucci et al.(7)



Bio-Rad Protein Assay Summary: Purification Process Tracking



Step Sample	Concentration (mg/ml)	Total Protein (mg)	Notes
10.00	10.00	4.000	~35% loss during desalting
6.25	6.25	2.500	Pooled 1, 2 & 3
1.80	1.80	0.900	
0.60	0.60	0.078	
Cumulative Summary			
Final Total Protein Yield	7.478 mg		Pooled Fraction Total 7.478 mg

Figure 5: SDS-PAGE analysis of pooled Ni-NTA elution fractions of His-tagged Syk kinase (6-269) in preparation for NMR foot printing of our best lead compounds. Four pools (one of many representative preps) were generated based on OD280 readings, with Pool 1 representing the highest concentration fractions.

References

1. Mócsai, A., Ruland, J. & Tybulewicz, V. L. J. The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat. Rev. Immunol.* **10,** 387–402 (2010).
2. D Bakthavatsalam, J.W. Craft Jr., *et al.*; Identification of Inhibitors of Integrin Cytoplasmic Domain Interactions With Syk; Front. Immunol., 08 January (2021)
3. Antenucci, L., Hytonen, V. P. & Ylanne, J. Phosphorylated immunoreceptor tyrosine-based activation motifs and integrin cytoplasmic domains activate spleen tyrosine kinase via distinct mechanisms. *J. Biol. Chem.* **293,** 4591–4602 (2018).
4. Woodside, D. G. *et al.* Activation of Syk protein tyrosine kinase through interaction with integrin Beta cytoplasmic domains. *Curr. Biol.* **11,** 1799–1804 (2001).
5. Woodside, D. G. *et al.* The N-terminal SH2 domains of Syk and ZAP-70 mediate phosphotyrosine-independent binding to integrin Beta cytoplasmic domains. *J. Biol. Chem.* **277,** 39401–39408 (2002).
6. Grädler, U. *et al.* Structural and biophysical characterization of the Syk activation switch. *J. Mol. Biol.* **425,** 309–333 (2013).
7. Antenucci, L., Hellman, M., Hytönen, V. P., Permi, P. & Ylänne, J. Integrin cytoplasmic domain and pITAM compete for spleen tyrosine kinase binding. *Front. Biosci.* **6, 8** (2019).

Acknowledgements

Grants: NIH/NHLBI R61/33 HL168737 (Woodside, Biediger, Craft). Microsoft CRM:0740135 (Craft). Center for Advanced Computing and Data Science Award SeFAC: 63445-B5006 (Craft), Summer Undergraduate Research Fellowship and Provost Undergraduate Research Scholarship (Merlinsky). University of Houston clusters (sabine and carya).