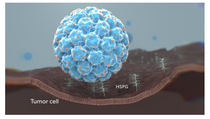
Abstract# 5331 Biological assessment of the virus-like drug conjugate AU-011 to specifically target a breadth of human cancer types

Rhonda C. Kines[1], Nathan R. Fons[2], Elisabet de los Pinos[1], John T. Schiller[2]
[1] Aura Biosciences, Cambridge, MA 02140 USA; [2] Laboratory of Cellular Oncology, NCI-NIH, Bethesda, MD 20892

Background

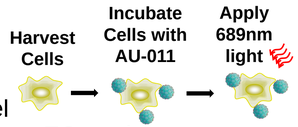


- Human papillomavirus virus-like particles (HPV VLP) preferentially target tumor cells via specifically modified heparan-sulfate proteoglycans (HSPG) on the cell surface.[1]
- AU-011 is an investigational virus-like drug conjugate composed of a modified HPV VLP and a near infrared light (nIR) activatable small molecule.[2]
- Upon activation with near infrared light (nIR), AU-011 causes acute tumor cytotoxicity *in vitro* and *in vivo*.[2,3]

Study Goal

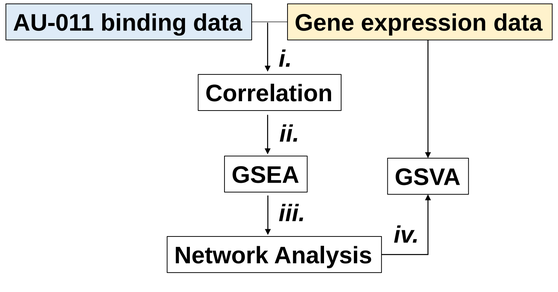
To explore the breadth of AU-011 efficacy on a comprehensive and diverse panel of 138 human cancer cell lines.

Methods



- *In vitro* binding and cytotoxicity of AU-011 was assessed using a panel of 138 human cancer cell lines *in vitro*. EC_{50} values were generated and the geometric mean fluorescent intensity (GMFI) of AU-011 binding across all dilutions was used to calculate the "Area Under the Curve" (AUC).
- Publicly available gene expression data for 115 cell lines was acquired from the Cancer Cell Line Encyclopedia (CCLE)[4] and was cross-referenced with the AUC values from the AU-011 binding panel to identify genetic correlates mediating AU-011 binding (i).
- Gene Set Enrichment Analysis (GSEA)[5] was performed on the dataset (ii), ranked based on Spearman rho (ρ) for each gene, with most significantly enriched gene sets used for Network construction[6] (iii).
- Gene Set Variant Analysis (GSVA)[7] was used to calculate enrichment scores (ES) on a per-cell line basis (iv) which were then used for downstream comparisons between cell line groups.

Analysis workflow

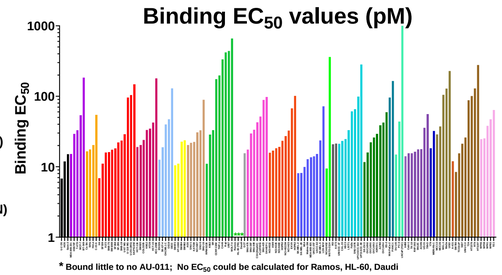


Tissue origin of cancer cell lines tested

Bone (M)	Mesothelioma (M)
Breast (E)	Muscle (M)
Cervix (E)	Oropharyngeal (E)
CNS (N)	Ovary (E)
Colon (E)	Pancreas (E)
Esophagus (E)	Prostate (E)
Fibrosarcoma (M)	Renal (E)
Gastric (E)	Retinoblastoma (N)
Hematopoietic (L)	Skin (MCC) (N)
Histiocytoma (M)	Skin (SCC) (E)
Liver (E)	Urothelial (E)
Lung (E)	Uveal Melanoma (N)
Melanoma (N)	

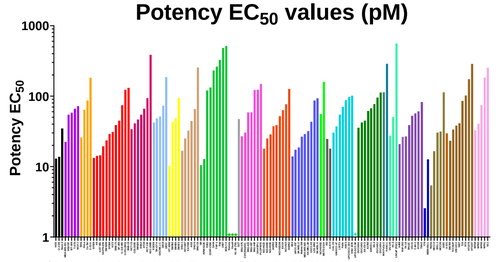
Developmental origin
E = Epithelial M = Mesenchymal
L = Lymphoid N = Neural

Binding EC_{50} values (pM)



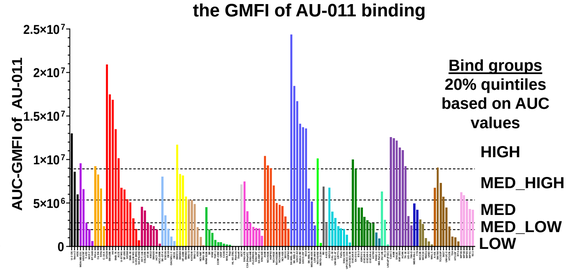
* Bound little to no AU-011; No EC_{50} could be calculated for Ramos, HL-60, Daudi

Potency EC_{50} values (pM)

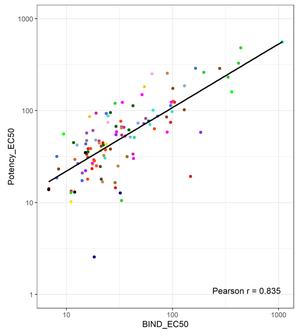


* EC_{50} could not be calculated for Ramos, HL-60, Daudi , UPCI-SCC-90

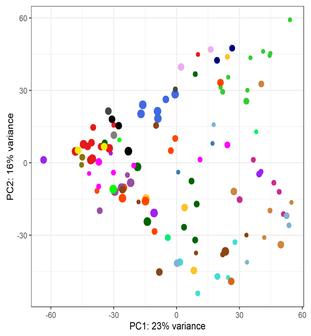
Area under the curve (AUC) calculated from the GMFI of AU-011 binding



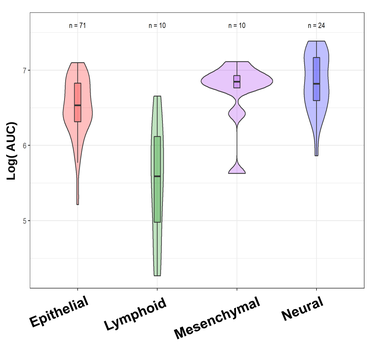
Binding vs. Potency (EC_{50}s)



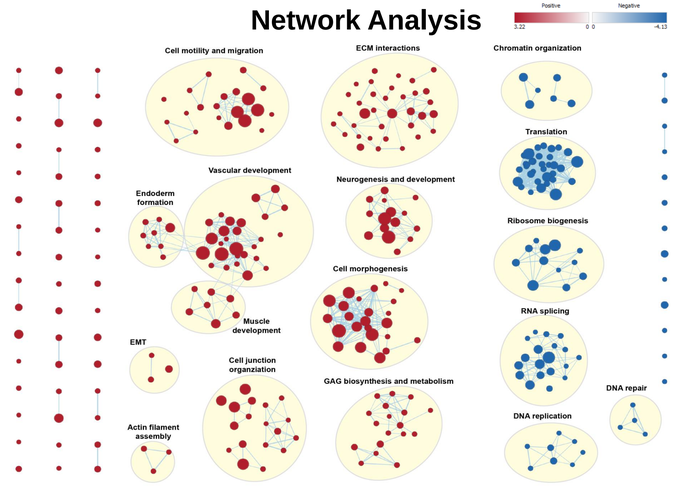
PCA-colored by Binding Quintiles



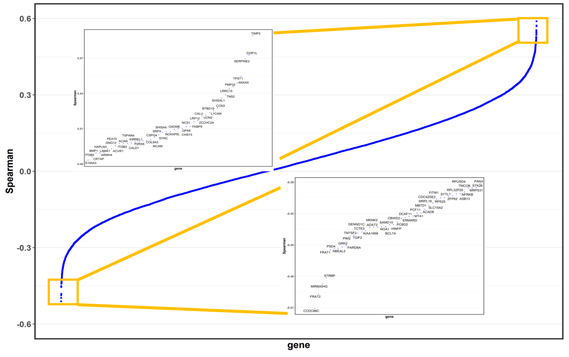
Binding based on Developmental Origin



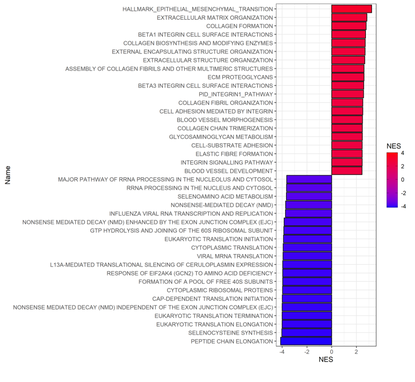
Network Analysis



Genetic correlates with AU-011 binding



Overview of Most Enriched Pathways



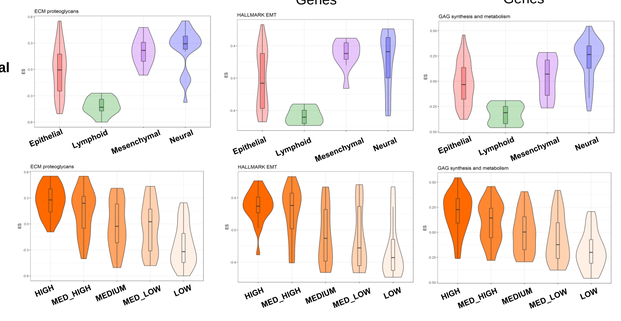
ES score of **Extra-Cellular Matrix Proteoglycan** Genes

ES score of **Epithelial-Mesenchymal Transition (EMT)** Genes

ES score of **GAG Synthesis and Metabolism** Genes

Developmental Origin

Binding Groups



References

1. Kines, R. and Cerio, R., et al. *Int. J Cancer*, 138(4):901-11, 2016.
2. Kines, R., et al. *Mol Cancer Ther*, 17(2):565-574, 2018.
3. Kines, R., et al. *Can Immunol Res* 9(6):693-706, 2021.
4. Ghandi, M., et al. *Nature* 569(7757): 503-508, 2019.
5. Subramanian, A., et al. *PNAS* 102(43): 15545-15550, 2005.
6. Reimand, J., et al. *Nature Protocols* 14(2): 482-517, 2019.
7. Hänzelmann, S. et al. *BMC Bioinformatics* 14(1): 1-15, 2013.

Conclusions

- Collectively these data demonstrate the wide potential applicability of AU-011 to target a number of tumor types, particularly those derived from neural or epithelial lineages.
- Correlative gene expression analysis demonstrated a strong association between AU-011 activity and genes involved in epithelial-to-mesenchymal transition, glycosaminoglycan biosynthesis/metabolism, and extracellular matrix interactions.
- Expression signatures for ribosomal activity and protein translation were negatively associated with AU-011 binding and activity.
- Importantly, a large portion of these tumors are accessible making their AU-011 targeting clinically translatable.