Exploring the Therapeutic Spectrum of Bevacizumab

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The Key Scientific Question (KSQ)

Using available scRNA-seq data from cancer cell lines, how can the use of the FDA-approved antibody therapy Bevacizumab be explored in additional cancers?

Bevacizumab Uses + Mechanism of Action

- Currently used for a variety of cancers, including colorectal, lung, breast and kidney cancer (Filis et. al 2010) and targets VEGF-A.
  - VEGF-A is a growth factor that is expressed under hypoxic conditions (the primary driver of angiogenesis)
  - VEGF-A receptors are on endothelial cells

- Bevacizumab acts by selectively binding to *circulating* VEGF-A, making it unable to bind to its receptors, leading to a reduction in blood supply to the tumor.

**A potential weakness of using cell lines (versus an in vivo model) to test bevacizumab is that cell lines cannot replicate angiogenesis, since these cells will not form new blood vessels.

Methodology

Phase 1

- Utilize *240701_kinker_anndata.ipynb* and *240702_kinker_scanpy.ipynb* provided by Dean Lee to process and visualize single cell RNA sequence data from various cancer cell lines in relation to cancers they indicate

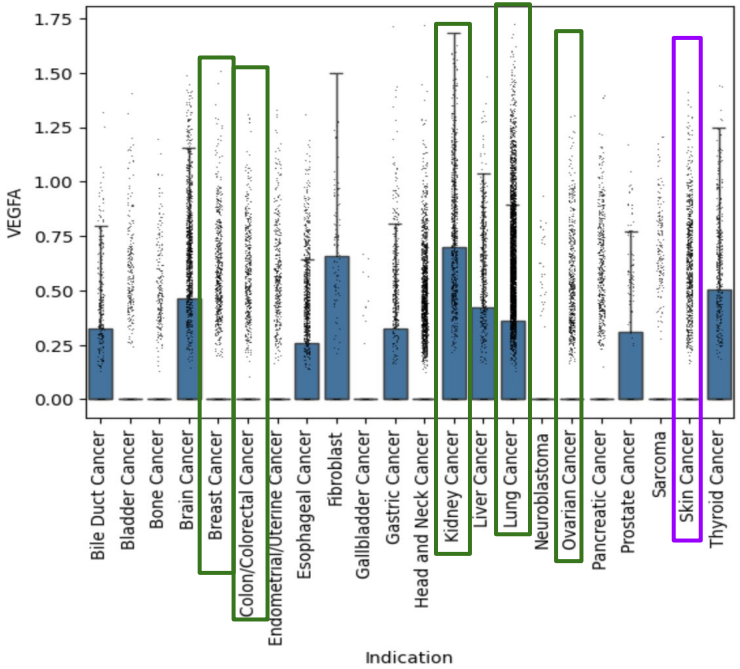
Phase 2

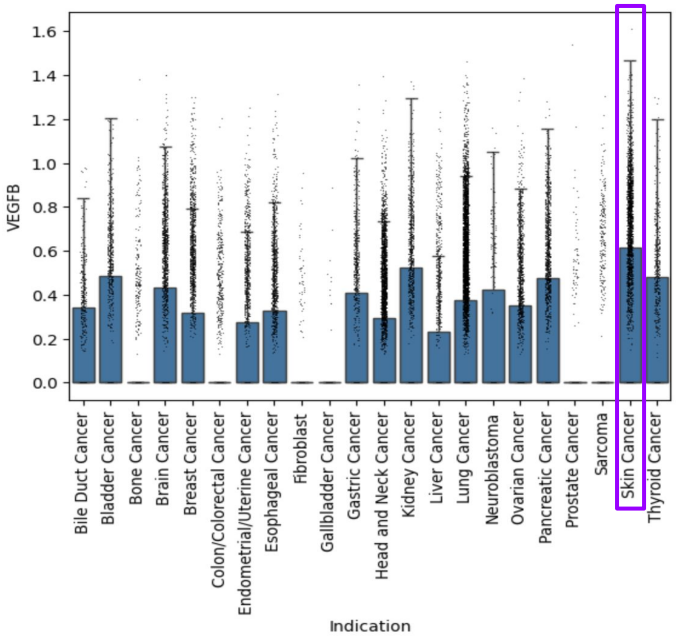
- Utilize *240703_kinker_explore.ipynb* provided by Dean Lee for analysis of VEGFA (gene encoding target protein of Bevacizumab) and VEGFB expression in different cancer cell lines

Phase 3

- Perform one-way ANOVA testing to determine significance of genetic expression, and pair genetic data with oncology literature

VEGF-A and VEGF-B Expression Related to General Cancer Indication

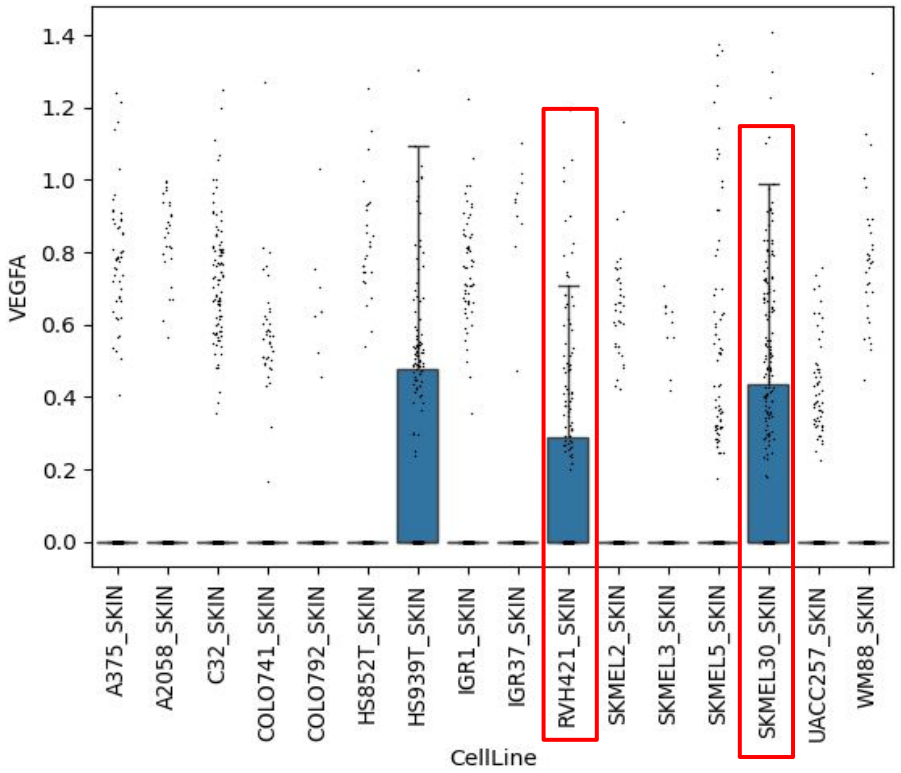




- Green boxes represent current FDA approved uses of Bevacizumab

- Given the prevalence of expression of VEGF-A and VEGF-B in the “Skin Cancer” indication (purple boxes), further analysis was conducted on skin cancer cell lines

VEGF-A expression in skin cancer cell lines



Red boxed cell lines = metastatic cutaneous melanoma

ANOVA

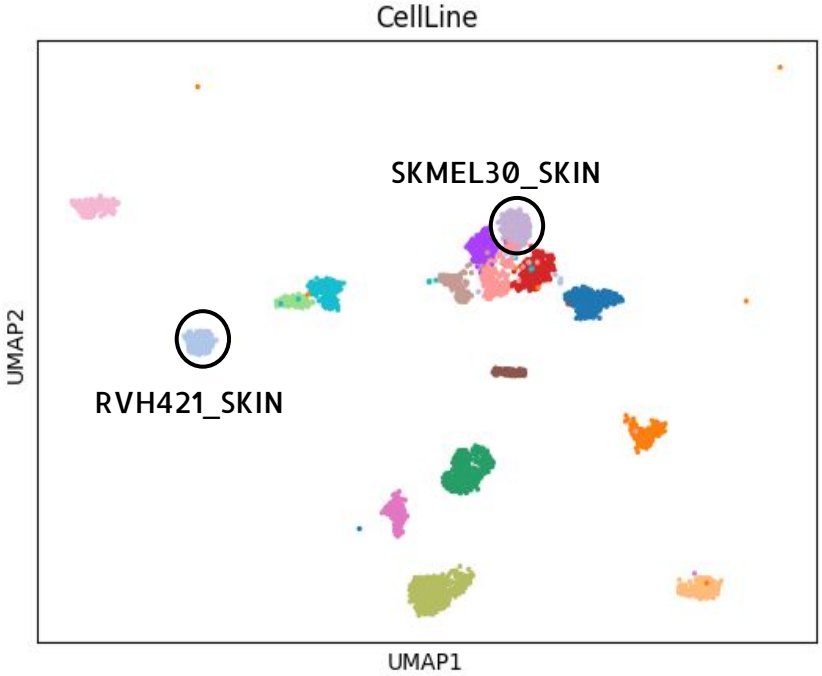
F-statistic — RVH421: 18.7

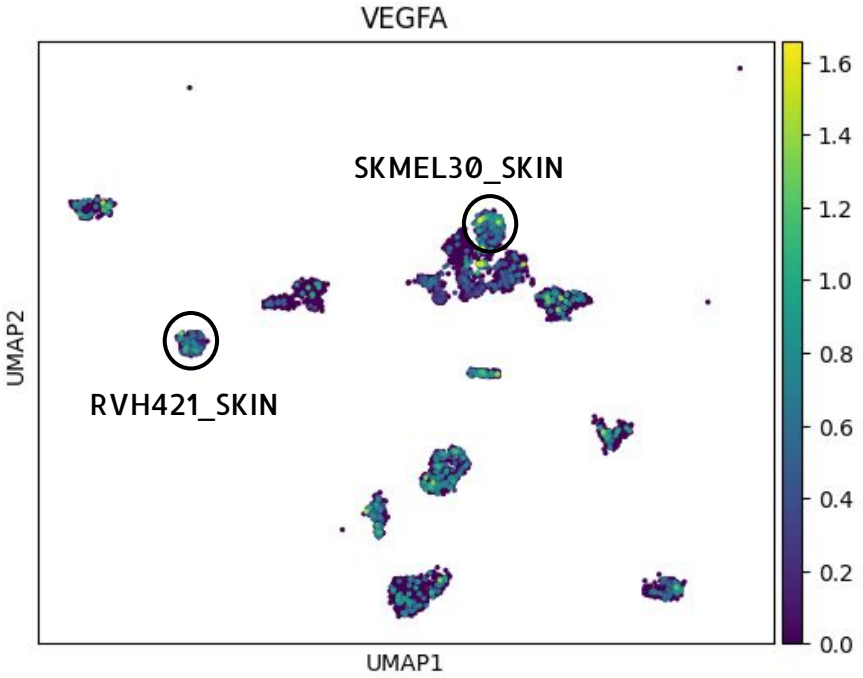
*Difference in expression is significant ($p < 0.05$) when compared to **50%** of all other skin cell lines*

F-statistic — SKMEL30: 18.7

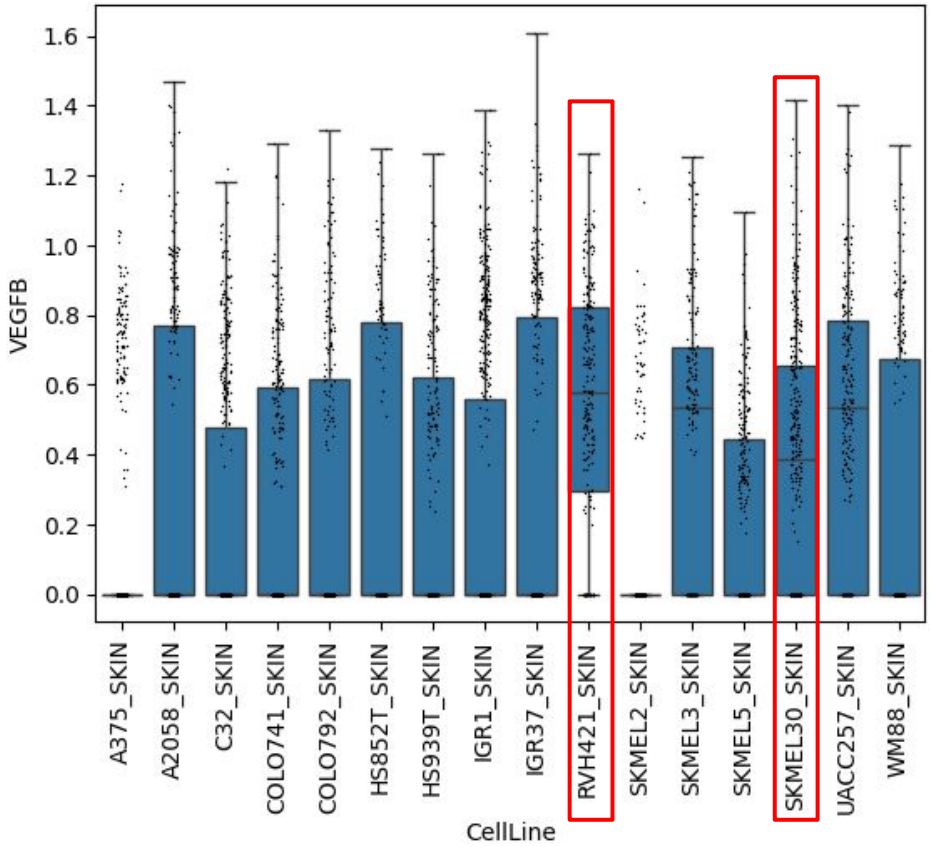
*Difference in expression is significant ($p < 0.05$) when compared to **86%** of all other skin cell lines*

VEGF-A expression in skin cancer cell lines





VEGF-B expression in skin cancer cell lines



ANOVA

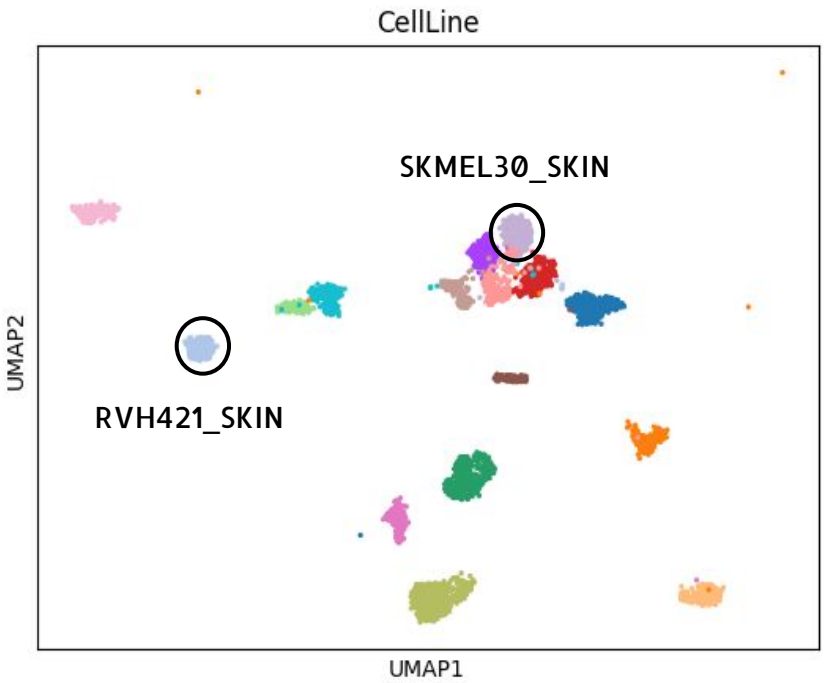
F-statistic — RVH421: 32.00

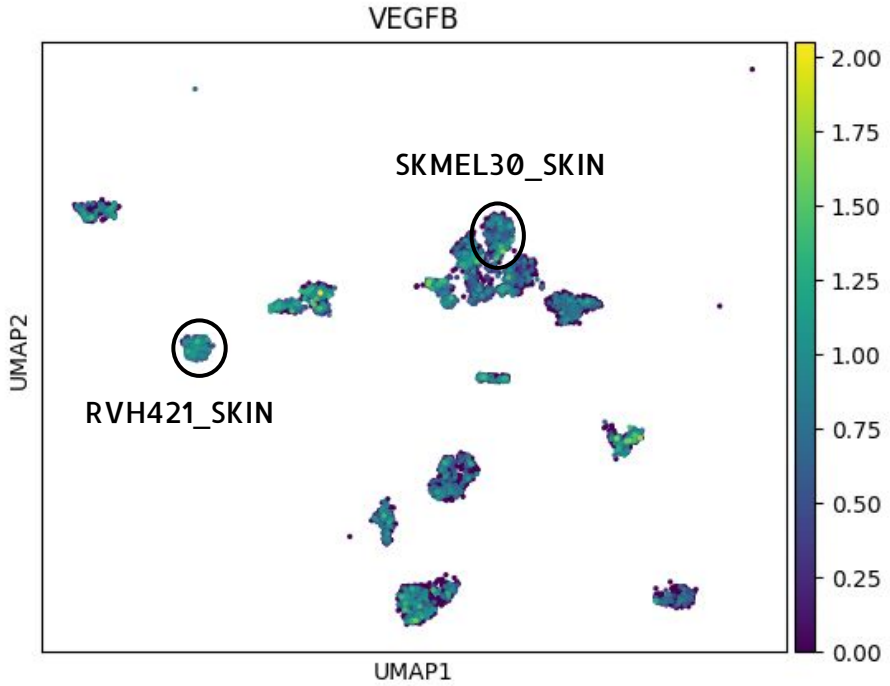
*Difference in expression is significant ($p<0.05$) when compared to **86%** of all other skin cell lines*

F-statistic — SKMEL30: 32.00

*Difference in expression is significant ($p<0.05$) when compared to **64%** of all other skin cell lines*

VEGF-B expression in skin cancer cell lines





Elevated VEGF-A *and* VEGF-B expression in metastatic cutaneous melanoma

- VEGF receptor expression is “one of the phenotypic changes occurring in melanoma cells during malignant transformation” (Gitagoryen et. al 2002), as these receptors are ***not*** present on normal melanocytes

- Rajabi et. al (2012) noted the expression of VEGF-A isoforms (both distribution and intensity) is “associated with progression of malignant melanoma”

- RVH421_SKIN and SKMEL30_SKIN are metastatic melanoma cell lines with elevated VEGF-A and VEGF-B gene expression, which have independent mechanisms to encourage metastasis (Yang et. al 2015)
  - VEGF-A = stimulates angiogenesis, increases vascular permeability, and enhances cancer cell invasion
  - VEGF-B = loss of perivascular cells to increase vascular leakiness, induces tumor hypoxia, and recruits M2-like tumor-associated macrophages to suppress immune system (Yang et. al 2015)

By targeting both VEGF-A and VEGF-B, melanoma metastasis could potentially be reduced

Utilizing alternating doses of B evacizumab and A flibercept in cutaneous malignant melanoma management

- Aflibercept is a decoy receptor with a high affinity for VEGF-A and VEGF-B and prevents the binding of VEGF-A and VEGF-B to native receptors (Eyewiki, 2024)

- Bevacizumab binds directly to VEGF-A, preventing receptor binding

- Strategy = Double down on VEGF-A targeting which directly encourages angiogenesis, and additionally target VEGF-B which promotes other metastatic processes

Targeting VEGF-A and VEGF-B, with additional efforts in targeting VEGF-A by using two drugs with different mechanisms may facilitate further reduction of metastasis

Next Steps

1. Use DESeq2 (a bioconductor package) for an additional analysis of differential genetic expression

2. Utilize in vivo models to test efficacy and toxicity of alternating doses of Bevacizumab and Aflibercept
   - This combination has been used in neovascular age-related macular degeneration, but not in cutaneous melanoma

3. Examine changes in angiogenesis/metastasis given this regimen

Data and Code Attribution

- Data analysis was built off Jupyter notebooks provided by Dean Lee from the F1L Internship Emulator Github repository

- SC-RNA data is from the Broad Institute's single-cell portal (SCP542) and at the Gene Expression Omnibus (GEO) (accession number GSE157220) from the work of Kinker et. al (2020) in "Pan-cancer single-cell RNA-seq identifies recurring programs of cellular heterogeneity"

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