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Is there more to intravitreal aflibercept than anti-angiogenesis? Evaluating additional effects in DME through an *in silico* approach

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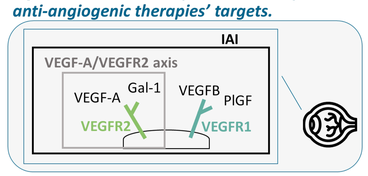
PURPOSE

- Diabetic macular edema (DME) is one of the **major causes of central vision and visual acuity loss** in diabetic patients,[1] involving various pathophysiological mechanisms, such as **angiogenesis, blood-retinal barrier (BRB) alteration and increased permeability, oxidative stress and inflammation.**[2]
- Vascular endothelial growth factor receptor 1 (**VEGFR1**) and 2 (**VEGFR2**) mediate signaling from activators identified as a key angiogenic factors up-regulated in ischemic retinopathies: **VEGF-A**, **VEGF-B**, placental growth factor (**PlGF**) and Galectin-1 (**Gal-1**).[3, 4]

Figure 1: Schematic representation of IAI and anti-angiogenic therapies' targets.



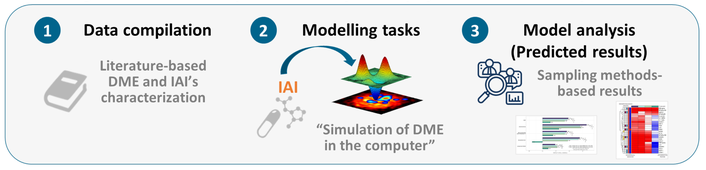
- Emerging evidence suggests that **VEGFR-1** also plays a crucial role in other pathophysiological mechanisms involved in DME such as **inflammation.**[5]
- Therapies targeting **VEGF-A/VEGFR2 axis are first-line treatments** for DME, among them, is the **intravitreal aflibercept injection** (IAI).[6]
- IAI presents a unique target profile that also includes the **inhibition of key ligands involved in VEGFR1 signaling** (Figure 1), suggesting a superior effect on DME compared to VEGF-A/VEGFR2-only inhibitors through its potential anti-inflammatory activity, as shown in preclinical studies.[7,8]

OBJECTIVE: TO EXPLORE THE MECHANISMS OF ACTION OF IAI ON THE PATHOPHYSIOLOGY OF DME BEYOND ANTI-ANGIOGENIC EFFECTS BY CREATING AN *IN SILICO* DME MODEL.

METHODS

The steps followed are represented in **figure 2**.

Figure 2: Schematic representation of the steps followed in this study.



DME and IAI characterization and DME model definition

- **Literature-based** DME and drug characterization.
  - PubMed database,[9] European Medicines Agency,[10] the Food and Drug Administration,[11] and product monographs; target information from specialized databases, such as Drugbank,[12] Stitch[13] and Supertarget.[14]
- **DME model: 4 pathophysiological processes** (oxidative stress, inflammation, BRB alteration and increased vascular permeability, and angiogenesis) were defined at protein level (**Table 1**).

Table 1. Summary of the processes identified as involved in DME. The number of protein effectors identified in each process are noted.

Process type	Process name	#Proteins
Causative	Oxidative stress	18
	Inflammation	33
	Alteration of BRB and increased vascular permeability	83
	Angiogenesis	37

Modelling: Therapeutic Performance Mapping System and sampling methods

- We applied Anaxomics proprietary **Therapeutic Performance Mapping System (TPMS)** technology[15] (systems biology-based machine learning approach) to analyze the impact of IAI on DME.
- The following scenarios were applied in DME model:
  - **IAI**: Inhibition of IAI targets (VEGF-B, PlGF, VEGF-A, Gal-1) and VEGFR1 and VEGFR2.
  - **VEGFR1-**: Inhibition of VEGFR1, and VEGF-B and PlGF ligands.
  - **VEGFR2-**: Inhibition of VEGFR2, and VEGF-A and Gal-1 ligands.
  - **VEGFR2-(DME)**: Inhibition of VEGFR2 pathway, including VEGF-A and Gal-1 ligands, while maintaining VEGFR1 activated, including VEGF-B and PlGF (inhibition of VEGFR2 pathway in the context of DME).

Analysis

- **Predicted protein activity and full signal** (derived from the TSignal[15] considering the whole response set, with a range of -1 to 1) were used to analyze pathophysiological processes and individual proteins.
- A **Mann–Whitney U test** was used for statistical analysis.

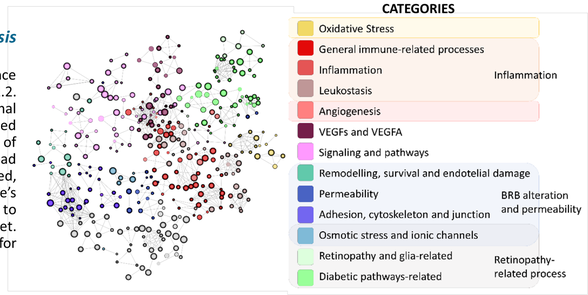
RESULTS

Definition of DME and IAI models

- We conducted an enrichment analysis based on all model proteins with predicted protein activity absolute value levels >0.8, observing protein sets from molecular pathways that could be broadly classified into the following categories of processes related to DME pathophysiology (**Figure 3**).

Figure 3: Enrichment analysis of DME model proteins

The maximum Hausdorff distance between protein sets was set at 1.2. The size of the node is proportional to the number of proteins included in the represented set. The color of the node illustrates the broad process in which each set is involved, and the thickness of the node's contour is inversely proportional to the FDR obtained for the set. Cytoscape v.3.9.1 was used for network representation.



IAI impact over defined DME pathophysiological processes

- Co-inhibition of VEGFR1 and VEGFR2 pathways by IAI treatment **affected all the pathophysiological processes associated with DME.** The strongest effects were seen on **angiogenesis, BRB alteration and permeability, and inflammation** (**Figure 4**).
- The sole inhibition of VEGFR1 or VEGFR2 pathways appears to be effective in treating DME:
  - **Angiogenesis** and **oxidative stress** are slightly more impacted by the inhibition of **VEGFR2**.
  - **BRB alteration and permeability** is slightly more affected by the inhibition of **VEGFR1**.
  - **Inflammation** is more regulated by **VEGFR1** signaling, and this process appears as the most benefited by the inclusion of VEGFR1 signaling inhibition in DME therapy (**IAI**).
- The model showed that the **activation of VEGFR1 signaling limits VEGFR2-(DME) treatment effectiveness in all processes,** and IAI outperforms it.

The results suggest that besides the inhibition of VEGFR2, **the inhibition of VEGFR1 pathway through IAI is crucial for the effective treatment of DME**, especially in the **inflammatory process**.

Molecular mechanism over the inflammatory process after IAI treatment in DME

- Co-inhibition of both VEGFR signaling pathways by **IAI** treatment contributes to the **reversion of the pathological status of most of the inflammatory effectors** (**Figure 5**).
- Inhibition of **both VEGFR1 and VEGFR2 receptors** modulates **p38 alpha and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathways** and **reinforces the impact over the inflammation process.**
- The inhibition of **VEGFR1** signaling pathway results in a deeper effect on the modulation of **E-selectin, interleukin-1 α (IL-1 α), IL-1 β, vascular cell adhesion protein-1 (VCAM-1), stromal cell derived factor-1 (SDF-1), IL-6, tumor necrosis factor α (TNF-α), IL-8, C-C motif chemokine 5 (CCL5)** when compared with VEGFR2 inhibition and VEGFR2-(DME) treatment.
- With **VEGFR2-(DME)** treatment, some of the effectors were kept in a **pathological state**, such as **TNF-α** and **IL-8**.

Inflammation was predicted to be the most impacted process by **VEGFR1 signaling inhibition,** modulating **leukostasis and cytokine-related protein effectors**.

Figure 4: Analysis of IAI and VEGFR impact over defined DME pathophysiological processes.

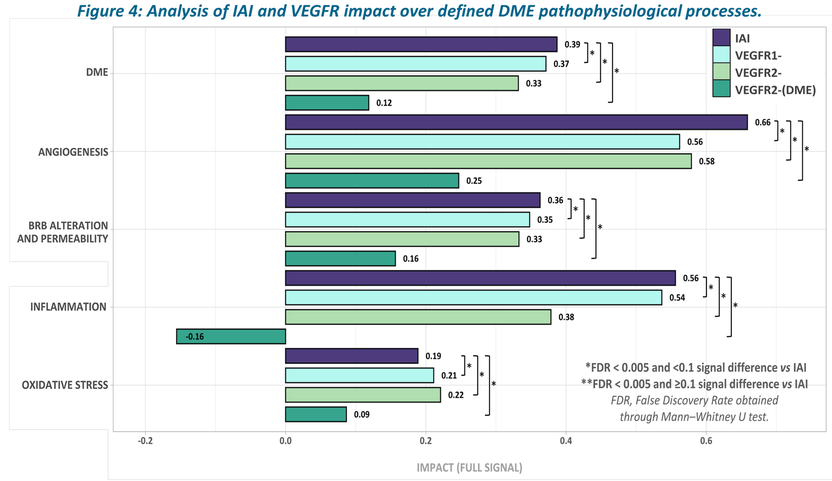
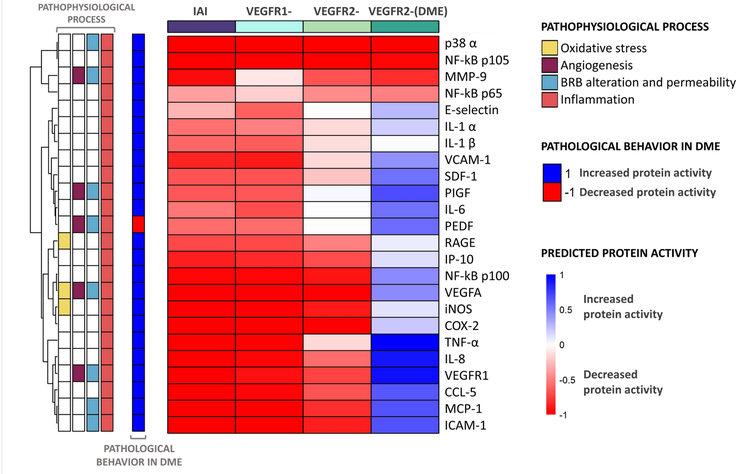


Figure 5: Heatmap representation of predicted activity of DME inflammation protein effectors.



CONCLUSIONS

- ✓ The constructed models, using the TPMS systems biology-based machine learning approach, **simulated the pathophysiology of DME and the mechanisms of action of IAI** via inhibition of VEGFR1 and VEGFR2 signaling.
- ✓ **IAI modulated all the pathophysiological processes associated with DME** and its strongest effects were observed not only over angiogenesis, but also over **BRB alteration and permeability, and inflammation.**
- ✓ **Inhibition of VEGFR1** signaling pathway results in a **deeper effect on the modulation of inflammatory proteins** when compared with VEGFR2 inhibition alone.
- ✓ Given the role of VEGFR1 signaling on the modulation of pathways related to inflammation, **IAI may offer therapeutic advantages through sustained VEGFR1 pathway inhibition**.

LIMITATIONS

- The study is focused on the main processes reported in the literature to be involved in DME as well as on the annotated knowledge around the targets evaluated, hence a bias towards better described pathways might occur.
- Given the complexity of VEGFA interaction with VEGFR1 and VEGFR2, it was assumed in this study that VEGFA-VEGFR1 signaling was irrelevant since it is limited by VEGFR1 solubility status and VEGFB or PlGF presence. Thus, our results assume VEGFR2 inhibition, while potentially underscoring the effects of drugs targeting VEGFA.
- Oxidative stress signaling depends largely on non-protein species, therefore, some oxidative stress-related signaling may not be reflected in a protein-based model.

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Disclosures

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