iLite® VEGF
ASSAY READY CELLS

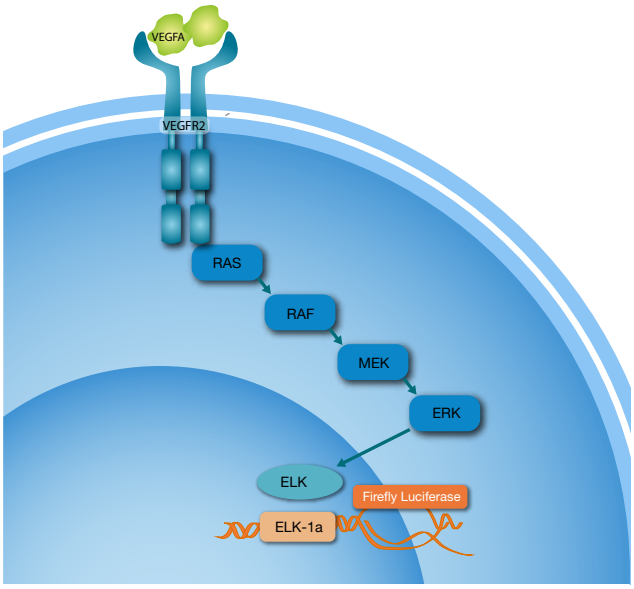
***iLite* VEGF Assay Ready Cells have been genetically engineered to specifically measure the activity of VEGF, the potency of VEGF inhibitors, and can also be used to detect neutralizing antibodies against inhibitor drugs in human serum.**

The signaling protein Vascular Endothelial Growth Factor (VEGF) is a major contributor to angiogenesis in both* health and disease. Since angiogenesis is required for tumour growth and metastasis, drugs targeting VEGF have been increasingly employed in a vast number of cancer therapies. In recent years, anti-VEGF therapies have also expanded into the area of ophthalmology, with several anti-VEGF drugs approved for retinal diseases.

The *iLite* VEGF Assay Ready Cells have been genetically engineered to specifically detect VEGF activity, and can be used to measure the potency of VEGF inhibitor drugs, as well as neutralizing antibodies against such drugs in human serum.

The *iLite* VEGF Assay Ready Cells measures the functional activity of VEGF and is not dependent on the structure of the drug, therefore, different anti-VEGF drugs can be measured in the same assay. As such, *iLite* VEGF Assay Ready Cells are well suited for direct comparisons of biosimilars and innovator drugs in the same assay, making them an excellent tool for biosimilar development.

** normal vascular growth and pathological scenarios*



By use of genome editing, only the relevant signaling pathway is activated by VEGF binding, leading to very high specificity.

- Highly sensitive
- Allows anti-VEGF activity to be quantified rapidly and in a highly specific manner
- Allows direct comparison between biosimilar and innovator drugs in the same assay

***iLite*® VEGF Assay Ready Cells**	
Product code	BM4021
Format	Assay Ready Cells
Related Products	BM4050 *iLite*® GM-CSF Assay Ready Cells BM3071 *iLite*® FGF21 Assay Ready Cells
Application	The *iLite* VEGF Assay Ready Cells can be used for the quantification of VEGF activity, VEGF inhibitor activity and for determination of neutralizing antibodies against VEGF inhibitors in human serum. Application notes for the following assays are available: • Quantification of functional VEGF using *iLite* VEGF Assay Ready Cells • Quantification of VEGF inhibitor activity using *iLite* VEGF Assay Ready Cells • Determination of neutralizing antibodies against VEGF inhibitors using *iLite* VEGF Assay Ready Cells
Incubation time	18 hours
Detection system	Luminescence
Availability	Research Use Only (RUO)*

**These products are intended for professional research use only. The data and results originating from using the products, should not be used either in diagnostic procedures or in human therapeutic applications.*

In accepting delivery of iLite® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third party, and recipient is only to use them directly in assays. The iLite® cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered iLite® Assay Ready Cells would constitute an infringement.

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Quantification of Bevacizumab Activity and Anti-Bevacizumab Neutralizing Antibodies in a Cohort of Patients with Glioblastoma

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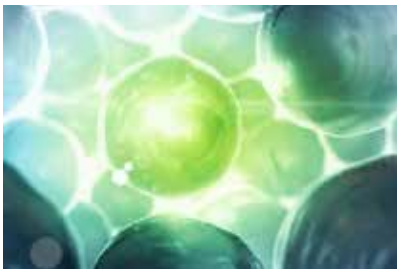


Abstract

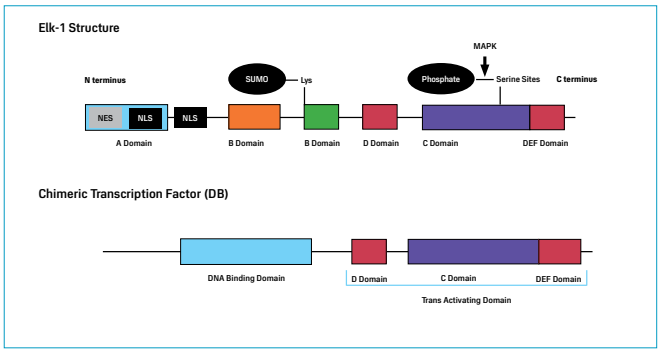
A novel highly sensitive VEGF-responsive reporter gene assay has been developed that allows bevacizumab activity to be quantified rapidly and in a highly specific manner. The use of this assay has shown that in a cohort of patients with glioblastoma who respond to therapy there is a close correlation between bevacizumab drug levels determined by ELISA and bevacizumab activity determined using the VEGF-responsive reporter gene assay. In contrast, in secondary non-responders with a decreasing PK profile, bevacizumab drug levels determined by ELISA are consistently higher than bevacizumab activity determined using the reporter gene assay, suggesting that bevacizumab activity is partially neutralized by anti-drug neutralizing antibodies (NAbs). The results obtained suggest that the use of the VEGF-responsive reporter gene assay may allow the appearance of anti-bevacizumab NAbs to be used as a surrogate maker of treatment failure prior to the clinical signs of disease progression.

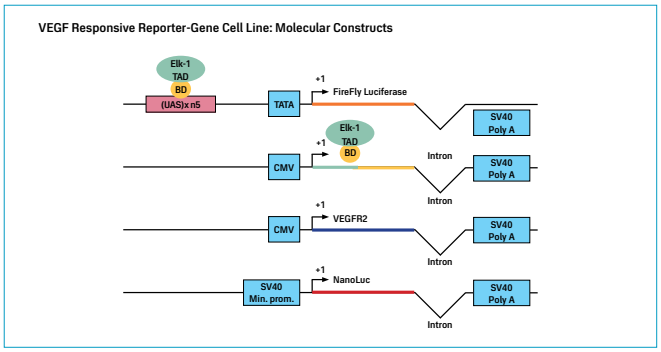
Introduction

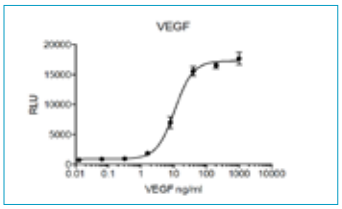
Vascular Endothelial Growth Factor (VEGF) plays a critical role in both normal vascular growth and pathological angiogenesis and the humanized anti-VEGF antibody bevacizumab (Avastin™, Genentech) is used widely as a first-line therapy for colorectal cancer and other neoplastic diseases. Current methods for quantifying the activity of human VEGF, or antibodies that neutralize its activity, are bioassays based on the ability of anti-VEGF antibodies to inhibit the proliferation or migration of primary human umbilical vein endothelial cells (HUVEC) or other cells expressing VEGR receptors, following treatment of the cells with VEGF. Such assays can take several days to perform, are subject to a high degree of variation, and are difficult to validate. Reporter-gene assays based on the establishment of a stable cell line transfected with a luciferase reporter-gene placed under the control of a drug responsive chimeric promoter, provide highly sensitive and reproducible methods for quantifying drug activity.

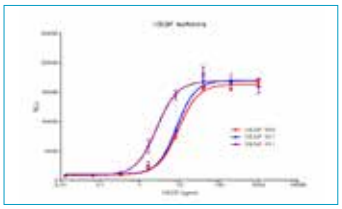
Methods

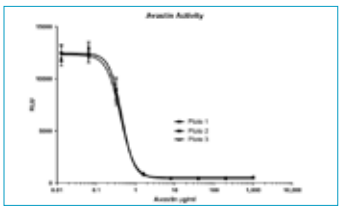
The VEGF family of proteins bind to tyrosine kinase receptors (VEGFR's) on the cell surface resulting in receptor dimerization and activation of a variety of kinases including MAPK2/3 and ERK1/2 and activation of transcription factors including Elk-1. In order to reduce the time required for a conventional VEGF bioassay using primary human cells, from 4 days or more to 24 hours or less and to obviate non-specific activation by other growth factors with overlapping biological activity human HEK293 cells were transfected with a chimeric transcription factor consisting of the trans-activating domain of Elk-1 fused to a synthetic DNA binding site and a reporter gene construct that responds specifically to the chimeric Elk-1 transcription factor.

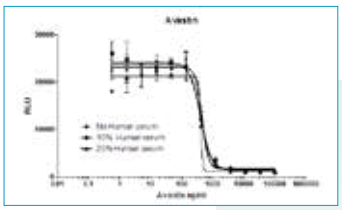


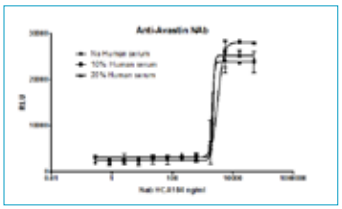


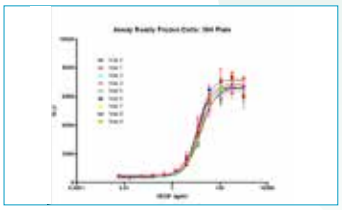


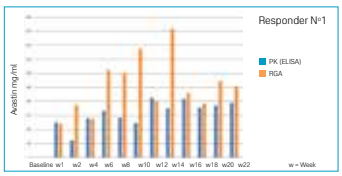


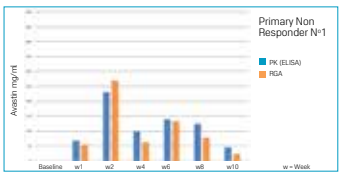


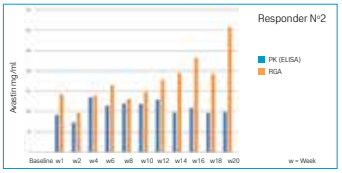


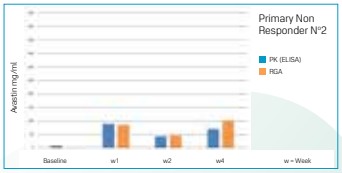


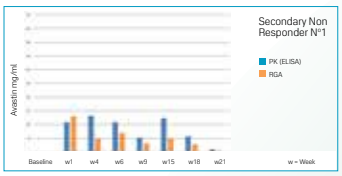


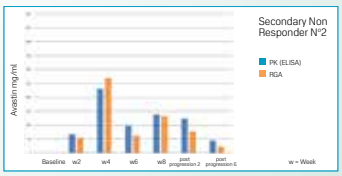












Conclusion

A good correlation between bevacizumab drug levels determined by ELISA and bevacizumab activity determined using a VEGF-responsive reporter gene assay was observed in patients with glioblastoma who respond to therapy. In contrast, in secondary non-responders with a decreasing PK profile bevacizumab drug levels determined by ELISA are similar or higher than bevacizumab activity suggesting that bevacizumab activity is partially neutralized by anti-drug neutralizing antibodies (NAbs). Additional studies are necessary in order to determine whether the use of the VEGF-responsive reporter gene assay will provide a means of detecting the early appearance of anti-bevacizumab NAbs as a surrogate maker of treatment failure.