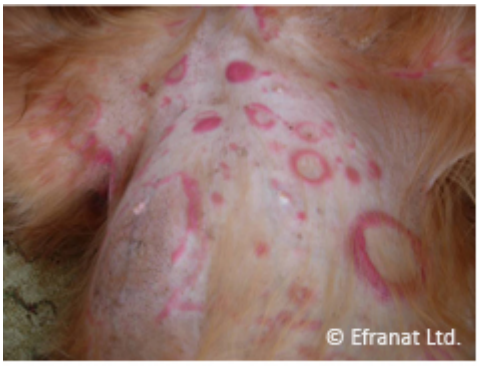
Canines Treatment Results

Efranat conducted compassionate, pre-clinical treatments of spontaneous cancer in canines at The Koret School of Veterinary Medicine, Hebrew University of Jerusalem (Beit Dagan). The research was approved by an ethics committee and Efranat received full consent from the canines' owners. The participating canines had life expectancies of a few months.
The study was led by Dr. Gillian Dank, a canine oncologist. All dogs underwent a variety of conventional therapies, such as tumor resection and chemotherapy, prior to initiating the EF-022 therapy. The recruited dogs were non-responsive or stopped responding to standard treatment.
14 canines were treated with EF-022 - SC injection at a dose of 0.5-1 ml/70 kg (200ng/ml EF-022) once a week.

One example:

Complete response of canine with recurring T cell cutaneous lymphoma, progress during EF-022 therapy



Before treatment

After third dose - complete response

2 years follow-up

Summary of the Results:

2/14 canines were fully recovered

7/14 canines responded to the treatment - partial response[1] or stable disease[2]

5/14 canines did not respond to the treatment. Those 4 canines had progressive disease[3] and died 4-6 weeks from the beginning of treatment

1) Partial response = >50% but <100% decrease in tumor size
2) Stable disease = tumor volume did not decrease by >50% or increase by >25%
3) Progressive disease = an increase in tumor volume of >25% or the appearance of new neoplastic lesions

Having received promising results from this study and other studies, Efranat has taken steps towards Phase 1 studies in cooperation with a leading medical center in Israel. The Company has also applied to the relevant regulatory authorities.

Technology

Efranat develops a unique anti-cancer immunotherapy treatment based on a natural plasma glycoprotein molecule that activates the innate immune system against cancer cells. This modified glycoprotein is a very potent immunomodulator that activates macrophages against cancer cells; however, its activity is remarkably reduced in patients with cancer. Efranat's drug, EF-022, is manufactured and processed under GMP in a procedure that mimics the natural process in the body. Administration of the drug bypasses the impaired macrophage activation cascade and efficiently activates macrophages against cancer.

Modified Vitamin D binding Protein Macrophage Activator

EF-022 is a modified version of vitamin D binding protein, glycoprotein found to activate macrophages. The technology is based on early research published by different scientists and was further developed by Efranat.
It is known that activated macrophages have potential to kill and eradicate cancer cells (11, 12). In 1991, it was found that macrophage activation requires serum vitamin D binding protein (VDBP also known as Gc protein) and participation of B and T lymphocytes (1). An inflammation-initiated macrophage-activation cascade was proposed where VDBP is deglycosylated by B and T cell membranous enzymes. VDBP carries a trisaccharide composed of N-acetylgalactosamine with dibranched galactose and sialic acid termini. VDBP is first hydrolyzed by the membranous b-galactosidase of B cells to yield a macrophage proactivating factor. This in turn is hydrolyzed by the membranous Neu- 1 sialidase of T-cells to yield the macrophage activating factor (MAF) which carries only monosacchride of N-acetylgalactosamine (2). Such selective deglycosylation is shown to occur naturally as part of the inflammatory response. In cancer patients the MAF precursor activity of the serum VDBP was found to be lost or reduced, since VDBP is deglycosylated by serum α-N-acetyl galactosaminidase (Nagalase) secreted from cancer cells (3). Deglycosylated VDBP protein without the N-acetylgalactosamine monoschride, cannot be converted to MAF, leading to immunosuppression. Administration of the vitamin D binding protein Macrophages Activator (VDBP-MAF) that was generated enzymatically in vitro from VDBP bypasses the impaired macrophage activation cascade and efficiently activates macrophages.

Vitamin D binding Protein Macrophage Activator Anti-cancer Activity

According to various publications, vitamin D binding protein Macrophages Activator (VDBP-MAF) is a potent anti-cancer agent. Systemic administration of VDBP-MAF to tumor bearing mice or hamsters inhibited the rate of tumor growth of various solid tumors and caused regression of established tumors (4, 5, 6, 7). It was found that in addition to its ability to activate tumoricidal macrophages, VDBP-MAF directly inhibits both proliferation and migration of cancer cells (8) and also angiogenesis. The antiangiogenic effects of VDBP-MAF is achieved by blocking critical steps such as human endothelial cell (HEC) proliferation, migration, tube formation and micro-vessel sprouting (9). Those effects may be mediated through the endothelial cells CD36 receptor (10) and by inhibiting VEGR-2 and ERK1/2 signaling cascades (9). VDBP-MAF angiogenesis inhibition was demonstrated in rat aortic ring and in chick embryo chorionallantoic membrane (CAM) assays (5, 9).
Efranat's product, EF-022, is modified version of the early studied molecule Vitamin D Binding Protein Macrophages Activator. Efranat developed a proprietary production process to enable GMP compliant manufacture of the product with improved stability and quality control analysis. The company unique product was found to be safe in toxicity study and showed good efficacy results in treating canines with advanced spontaneous tumors. Efranat initiated Ph1 study in 2014 and plans to continue the study in the course of 2015.

References

Identification of the serum factor required for in vitro activation of macrophages. Role of vitamin D3-binding protein (group specific component, Gc) in lysophospholipid activation of mouse peritoneal macrophages.

Yamamoto N, Homma S, Millman I.
J Immunol. 1991 *Jul 1;147(1):273-80.*

Conversion of vitamin D3 binding protein (group-specific component) to a macrophage activating factor by the stepwise action of beta-galactosidase of B cells and sialidase of T cells.

Yamamoto N, Kumashiro R.
J Immunol. 1993 *Sep 1;151(5):2794-802.*

Deglycosylation of serum vitamin D3-binding protein leads to immunosuppression in cancer patients.

Yamamoto N, Naraparaju VR, Asbell SO.
Cancer Res. 1996 *Jun 15;56(12):2827-31.*

Macrophage-directed immunotherapy as adjuvant to photodynamic therapy of cancer.

Korbelik M, Naraparaju VR, Yamamoto N.
Br J Cancer. 1997 *;75(2):202-7.*

Vitamin D binding protein-macrophage activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice.

Kisker O, Onizuka S, Becker CM, Fannon M, Flynn E, D'Amato R, Zetter B, Folkman J, Ray R, Swamy N, Pirie-Shepherd S.
Neoplasia. 2003 *Jan-Feb;5(1):32-40.*

Vitamin D binding protein-macrophage activating factor inhibits HCC in SCID mice.

Nonaka K, Onizuka S, Ishibashi H, Uto Y, Hori H, Nakayama T, Matsuura N, Kanematsu T, Fujioka H.
J Surg Res. 2012 *Jan;172(1):116-22. doi: 10.1016/j.jss.2010.07.057. Epub 2010 Sep 17.*

Inhibitory effect of vitamin D-binding protein-derived macrophage activating factor on DMBA-induced hamster cheek pouch carcinogenesis and its derived carcinoma cell line.

Toyohara Y, Hashitani S, Kishimoto H, Noguchi K, Yamamoto N, Urade M.
Oncol Lett. 2011 *Jul;2(4):685-691. Epub 2011 May 13.*

Vitamin D binding protein-macrophage activating factor directly inhibits proliferation, migration, and uPAR expression of prostate cancer cells.

Gregory KJ, Zhao B, Bielenberg DR, Dridi S, Wu J, Jiang W, Huang B, Pirie-Shepherd S, Fannon M.
PLoS One. 2010 *Oct 18;5(10):e13428. doi: 10.1371/journal.pone.0013428.*

Inhibition of angiogenesis by vitamin D-binding protein: characterization of anti-endothelial activity of DBP-maf.

Kalkunte S, Brard L, Granai CO, Swamy N.
Angiogenesis. 2005;*8(4):349-60. Epub 2006 Jan 7.*

Effects of vitamin D(3)-binding protein-derived macrophage activating factor (GcMAF) on angiogenesis.
Kanda S, Mochizuki Y, Miyata Y, Kanetake H, Yamamoto N.
J Natl Cancer Inst. 2002 *Sep 4;94(17):1311-9.*

The macrophage response to infectious agents: mechanisms of macrophage activation and tumour cell killing.
Keller R.
Res Immunol. 1993 *May;144(4):271-3; discussion 294-8. Review. No abstract available.*

Macrophage tumoricidal mechanisms.
Klostergaard J.
Res Immunol. 1993 *May;144(4):274-6. Review. No abstract available.*