

Novel Class of Turn-ON Near-Infrared Probes for Diagnosis and Imaging of Inflammation and Cancer (Ramot) code: 5-2012-286 <u>Ronit SATCHI-FAINARO</u>, T.A.U Tel Aviv University, Medicine-Sackler Faculty, Physiology and Pharmacology <u>Doron SHABAT</u>, T.A.U Tel Aviv University, Exact Sciences, School of Chemistry

Complete tumor removal during surgery has a great impact on patient survival. To that end, the surgeon should detect the tumor, remove it and validate that there are no residual cancer cells left behind. Residual cells at the incision margin of the tissue removed during surgery are associated with tumor recurrence and poor prognosis for the patient.

# **UNMET NEED**

There is an unmet need for advanced technology and diagnostic tools to better delineate tumor boundaries in real-time during surgery for complete tumor resection. This may reduce the risk for tumor recurrence, repeated surgeries and improve post-surgery quality of life due to minimal harm to healthy tissue. Hopefully, it will lead to improved patient survival rates.

## **OUR SOLUTION**

We devised a library of novel nano-sized polymeric Turn-ON probes that are activated at the tumor site by cysteine cathepsins that are highly expressed in multiple tumor types, out of which we chose 2 leading "smart" probes.

## **OUR PRODUCT**

We designed, synthesized and characterized two novel polymeric near infra-red fluorescent (NIRF) Turn-ON probes that are activated by cathepsins. These Turn-ON probes possess unique properties, resulting from different structures and activation modes for the application of image-guided surgery, which differentiate them from previously reported probes. The first polymeric Turn-ON probe (System 1) is based on HPMA copolymer and is activated by suitable linker degradation thus can be tailored according to the enzyme of choice. The second system (System 2) is based on PGA polymeric backbone, which is biodegradable by cysteine cathepsins. We characterized our conjugates in vitro and in vivo and found that both systems were stable in vitro in 50% mouse plasma, up to 100 h. In vivo PK studies revealed t1/2 of ~20 min for both systems. Finally, the in vivo tumor-to-background signal was above 2-4-folds higher than the signal obtained from the healthy tissue. Both systems are non-toxic at the concentrations used for in vitro and in vivo studies. There are several instruments that are available (FLARE, Solaris, SPY, etc.) for fluorescence imaging during surgical procedures. However, several of them need wavelength adjustment. FDA approved systems include: Fluoptics Fluobeam 800, Quest Spectrum, Novodaq SPY Elite, Hamamatsu PDE Neo, VisionSence Iridium.

#### DIFFERENTIATION

We designed two Turn-ON systems for image-guided surgery and detection applications. Currently, FDA approved 5-aminolevulinic acid (5-ALA, Gliolan, Medac GmbH) is most commonly used during glioblastoma excision surgeries. Another commercially available polymeric system for research only is ProSense 680 (by PerkinElmer). The Side-by-side comparison is summarized in Table 1 below.

Our study showed that the group that underwent surgery using HPMA copolymer-based system guidance demonstrated longer survival rates than the control groups that underwent either non-guided surgery, or surgery-guided by ProSense 680 or 5-ALA.

Our further study showed that surgery preformed by PGA-based diagnostic system, bearing a FRET pair, in the NIR range, followed by anticancer treatment with PGA based therapeutic system combining two targeted therapy agents, BRAF and MEK inhibitors decreased the recurrence of melanoma primary tumor, as well as metastases development and prolonged the survival of the

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Yeda Research & Development Co. Ltd, P.O Box 95, Rehovot 7610002, Israel, Telephone: 972-8-9470617, Fax: 972-8-9470739



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# Table 1:

System 1 (HPMA- based)	System 2 (PGA- based)	ProSense 680**	5-ALA*	
Time to surgery	4 h	3 h	24 h	3 h
Sensitivity <i>in vivo</i> (cancerous <i>vs</i> healthy tissue)	2.2-fold	4.2-fold	1.9	1.16
Sensitivity <i>in vitro</i> (with <i>vs.</i> without the enzyme)	2.6-fold	5.5-fold	-	-
Advantages	Modular***	Biodegradable	Biodegradable	Small molecule
Administration	IV	IV	IV	PO

# \*FDA approved

\*\*Research agent (by PerkinElmer), not FDA approved

\*\*\*System 1 is activated by cysteine cathepsins which are enzymes overexpressed in many tumor types. In addition, it can be modular and tailored according to the desired enzyme or analyte evaluated in the tumor site.

\*\*\*\*System 2 and ProSense 680 are biodegradable by cysteine cathepsins.

# PATENTS

Activatable fluorogenic compounds and uses thereof as near infrared probes. Patent number: 10071983. Type: Grant. Filed: April 18, 2016. Date of Patent: September 11, 2018. Assignee: Ramot at Tel-Aviv University Ltd. Inventors: Shabat, Satchi-Fainaro.

Polymeric systems and uses thereof in theranostic applications

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# REFERENCES

1. Blau R<sup>®</sup>, Epshtein Y<sup>®</sup>, Pisarevsky E<sup>®</sup>, et al. (2018). Image-Guided Surgery Using Near-Infrared Turn-ON Fluorescent Nanoprobes for Precise Detection of Tumor Margins. Theranostics, 8(13):3437-3460.

2. Ferber S�, Baabur-Cohen H�, Blau R�, et al. (2014). Polymeric nanotheranostics for real-time non-invasive optical imaging of breast cancer progression and drug release. Cancer Letters, 352(1): 81-89.

3. Blau R, Krivitsky A, Epshtein Y, Satchi-Fainaro R, Are nanotheranostics and nanodiagnostics-guided drug delivery stepping stones toward precision medicine?, Drug Resistance Updates, 27:39-58 (2016).

4. Ferber S, Tiram G and Satchi-Fainaro R, Monitoring functionality and morphology of vasculature recruited by factors secreted by fast-growing tumor-generating cells, The Journal of Visualized Experiments (JoVE), Nov 23;(93):e51525 (2014).



5. 43. Redy-Keisar O, Ferber S, Satchi-Fainaro R\* and Shabat D\*, NIR Fluorogenic Dye as a Modular Platform for Prodrug Assembly: Real-Time in vivo Monitoring of Drug Release, ChemMedChem, 10(6): 999-1007 (2015). \*Corresponding authors.

6. Redy-Keisar O, Kisin-Finfer E, Ferber S, Satchi-Fainaro R\*, and Shabat D\*, Synthesis and Use of QCy7-derived Modular Probes for Detection and Imaging of Biologically Relevant Analytes, Nature Protocols, 9(1), 27-36 (2014). \*Corresponding authors.

7. 39. Kisin-Finfer E, Ferber S, Blau R, Satchi-Fainaro R, Shabat D, Synthesis and evaluation of new NIR-fluorescent probes for cathepsin B: ICT versus FRET as a turn-ON mode-of-action, Bioorganic and Medicinal Chemistry Letters, 24(11):2453- 2458 (2014).

8. Karton-Lifshin N, Segal E, Omer L, Portnoy M, Satchi-Fainaro R\*, Shabat D\*, A Unique Paradigm for a Turn-ON Near-Infrared Cyanine-Based Probe: Non-Invasive Intravital Optical Imaging of Hydrogen Peroxide, Journal of the American Chemical Society (JACS), 133(28), 10960-10965 (2011). \*Corresponding authors.

9. Pogue W. Brian, et al. (2016). Review of fluorescence guided surgery systems: identification of key performance capabilities beyond indocyanine green imaging. J Biomed Opt. 21 (8): 080901.

## Contact for more information:

Ariela Markel 🖂, VP Business Development, Healthcare , 02-6586608

Ramot at Tel Aviv University Ltd. P.O. Box 39296, Tel Aviv 61392 ISRAEL Phone: +972-3-6406608 Fax: +972-3-6406675