Novel Class of Turn-ON Near-Infrared Probes for Diagnosis and Imaging of Inflammation and Cancer (Ramot)

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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

mice.

Table 1:

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

*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
Publication number: 20170014531. Type: Application. Filed: March 13, 2015.
Publication date: January 19, 2017. Inventors: Satchi-Fainaro, Shabat, Blau, Epshtein, Baabur-Cohen, Ferber, Redy-Keisar, Kisin-Finfer.

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