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Vascular Targeted Photodynamic Therapy (VTP): A New Modality that Cuts Off The Tumor Blood Supply

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In Photodynamic Therapy (PDT) a light beam is used to photosensitize a drug inducing cytotoxic processes with local tissue damage. PDT is currently used for local therapy of several cancer types and of ophthalmic age related macular degeneration. The specific anti-vascular strategy (VTP) developed in our laboratories is based on bacteriochlorophyll derivatives as sensitizing drugs targeted at the tumor blood vessels during a 5-15 min treatment session. VTP was shown efficient for treatment of various solid tumors (Table 1). This technique is presently under clinical trials for prostate cancer therapy in Canada, UK and Israel.

Research Objectives:

Our studies aim at further improvement of VTP for clinical use, development of new sensitizers and treatment applications and elucidation of the mechanistic basis of its actions at the molecular, cellular and systemic levels.

Research Achievements:

Online Imaging of the photodynamic process: VTP is based on *in-situ* photosensitization of the circulating drug leading to local generation of cytotoxic reactive oxygen species (ROS), which then causes rapid vascular occlusion, stasis, necrosis and tumor eradication. Intravascular ROS production is associated with photoconsumption of oxyhemoglobin (Hb) and consequent accumulation of paramagnetic deoxyHb. We evaluated the use of functional MRI for online monitoring of PDT efficacy. Using a solid tumor model, we demonstrated that Pd-bacteriopheophorbide (TOOKAD)-PDT significantly attenuates (25-40%) the MR signal, at the illuminated tumor site only. This phenomenon is independent of, though augmented by, ensuing changes in blood flow. The concept of photosensitized functional MRI may find intraoperative applications in guidance/monitoring of anti-vascular therapy (2). *(In collaboration with Michal Neeman)*

Destruction of the tumor vasculature is sufficient for tumor eradication: To firmly establish that destruction of the tumor vasculature is sufficient to induce tumor eradication TOOKAD-PDT was applied to a human HT29/MDR colon carcinoma tumor xenograft model consisting of multidrug resistant (MDR) cells that are not responsive to TOOKAD-PDT *in vitro*. Our results demonstrated that VTP induces tumor necrosis with equal efficacy (88 versus 82%) in the MDR variant and its wild type control counterpart. These results are ascribed to the antivascular effects of the treatment, supporting the hypothesis that MDR tumors can be successfully eradicated by indirect approaches bypassing their inherent drug resistance. We suggest that targeting the treatment to the vascular network, the lifeline of the tumor, is a promising alternative for the treatment of drug resistant tumors (3). *(In collaboration with Moti Liscovitch).*

Antivascular activity of WST11-VTP: Selective vascular destruction of human HT29 colon carcinoma xenografts by WST11 (a water soluble derivative of TOOKAD) was observed. Several techniques were used to characterize the vascular response to WST11-VTP, including intravital microscopy, use of vital dyes in animal studies, histology and immunohistochemistry. We found that WST11-VTP using a light dose of 100mW/cm^2 induces immediate, permanent and selective shutdown of the tumor vasculature. However, increasing the light dose to 200mW/cm^2 abolished the selective effect, and induce vascular shutdown of the healthy tissue as well. WST11-PDT induced a transient increase in the vessel permeability of the illuminated healthy tissue around the tumor, resulting in local edema. WST11-VTP induced tumor necrosis and flattening

accompanied by lipid peroxidation with a high (91%) cure rate (Table 1).

Early detection of response to VTP of human adenocarcinoma by diffusion MRI: We detected an unexpected decline in water diffusion coefficient at early times after VTP in the treated tumor. The study correlates this decline in diffusion-MRI with positive response of the tumor to VTP (local necrosis and decline in plasma PSA). Diffusion MRI of TOOKAD-VTP provides a unique signal that allows for the detection of tumor response to VTP within 7 hours in the live mouse and possibly also in patients (4). *(In collaboration with Michal Neeman)*

Hemodynamic changes induced by VTP at the treatment site as monitored by intravital video fluorescence microscopy (IVFM): In vivo imaging of the effect of TOOKAD-PDT and WST11-PDT on microcirculation and platelet/WBC endothelial interaction were studied using IVFM in the mouse ear model. VTP induced thrombus formation during the illumination period. Adhesion and detachment of platelets were seen most clearly in the venules, though blood flow stopped faster in the arteriols due to faster onset of occlusion. Adhered platelets appeared to detach, apparently due to increasing sheer force in the venules. Thrombi also contained fluorescently labeled leukocytes that were identified as relatively bright fluorescent spots. No changes in platelet/leukocyte-endothelial interaction nor thrombus formation were observed before the treatment or in control mice. Other studies showed induction of blood vessel permeability and vasoconstriction of arterioles (2). This technique allows for noninvasive online monitoring of VTP.

Studies in Cell cultures revealed the following results:

1. The major ROS photogenerated by TOOKAD were identified intracellularly as hydroxyl radicals and superoxide.
2. Properties of the EGF receptor were found to be modified by WST11-PDT.
3. Adaptation to oxidative stress induced by WST11 was studied.
4. Serum albumin was found to be a major factor mediating uptake of WST11 by cells.
5. TOOKAD-PDT activates cellular caspases but cell-killing is caspase-independent.

Table 1. Xenograft models and cure rate with bacteriochlorophyll based photosensitizers

Tumor type / photosensitizer / animal model	Cure rate1 %
Rat C6 glioma/TOOKAD/CD1 nude mice	64
Human small cell carcinoma of the prostate/ TOOKAD/CD1 nude mice	69
Human adenocarcinoma of the prostate /TOOKAD/CD1 nude mice	742
Human HT29 colon carcinoma/ WST11/ CD1 nude mice	91
Canine Transmissible Venereal tumor /TOOKAD/CD1 nude mice	83
Human HT29 colon carcinoma WT/MDR+/TOOKAD/CD1 nude mice	82/88[2]
Rat DS sarcoma3 / Wistar rat	78
Mouse M2R melanoma3 / CD1 nude mice	80

1. Single treatment session; 2. Tumor necrosis as endpoint.
3. Bacteriochlorophyll-serine as photosensitizer.

Selected Publications

Koudinova, N, Pinthus, J.H. , Brandis, A., Brenner, O., Bendel, P., Ramon J., Eshhar, Z., Scherz, A, Salomon, Y. (2003), Intl. J. Cancer, 104, 782-789.

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Plaks, V., Koudinova, N., Nevo, U., Pinthus, J.H., Kanety, H., Eshhar, Z., Ramon, J., Scherz, A., Neeman M. and Salomon, Y. (2004), Neoplasia, in Press.

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