Experimental set-up for FLASH proton irradiation of small animals using a clinical system

Summary

Purpose

Recent in vivo investigations have shown that short pulses (FLASH) of electrons are less harmful to healthy tissues, but just as efficient as conventional dose-rate radiation to inhibit tumor growth. In view of the potential clinical value of FLASH and the availability of modern proton therapy infrastructures to achieve this goal, we herein describe a series of technological developments required to investigate the biology of FLASH irradiation, using a commercially available clinical proton therapy system.

Methods and materials

Numerical simulations and experimental dosimetric characterization of a modified clinical proton beamline, upstream from the isocenter were performed with Monte Carlo toolkit and different detectors. A single scattering system was optimized together with a ridge filter and a high current monitoring system. In addition, a submillimetric set-up protocol based on image-guidance using a digital camera and an animal positioning system was also developed.

Results

The dosimetric properties of the resulting beam and monitoring system were characterized: linearity with dose rate and homogeneity for a 12×12 mm² field size were assessed. Dose rates exceeding 40 Gy/s at energies between 138 and 198 MeV were obtained, enabling uniform irradiation for radiobiology investigations on small animals in a modified clinical proton beam line.

Conclusion

This approach will enable us to conduct FLASH proton therapy experiments on small animals, specifically for mouse lung irradiation. Dose rates exceeding 40 Gy/s were achieved, which was not possible with the conventional clinical mode of the existing beamline.

Marie-Catherine Vozenin, Pauline De Fornel, Kristoffer Petersson, Vincent Favaudon, Maud
Jaccard, Jean-François Germond, Benoit Petit, Marco Burki, Gisèle Ferrand, David Patin, Hanan Bouchaab, Mahmut Ozsahin, François Bochud, Claude Bailat, Patrick Devauchelle, Jean Bourhis (2018 Jun 8)

**The Advantage of FLASH Radiotherapy Confirmed in Mini-pig and Cat-cancer Patients.**


**Summary**

Previous studies using FLASH radiotherapy (RT) in mice showed a marked increase of the differential effect between normal tissue and tumors. To stimulate clinical transfer, we evaluated whether this effect could also occur in higher mammals.

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Sylvain Thierry, Wael Jdey, Solana Alculumbre, Vassili Soumelis, Patricia Noguiez-Hellin, Marie Dutreix (2017 Sep 27)

**The DNA repair inhibitor Dbait is specific for malignant hematologic cells in blood.**


**Summary**

Hematologic malignancies are rare cancers that develop refractory disease upon patient relapse, resulting in decreased life expectancy and quality of life. DNA repair inhibitors are promising strategy to treat cancer but are limited by their hematologic toxicity in combination with conventional chemotherapies. Dbait are large molecules targeting the signaling of DNA damage and inhibiting all the double-strand DNA break pathways. Dbait have been shown to sensitize resistant solid tumors to radiotherapy and Platinium salts. Here, we analyze the efficacy and lack of toxicity of AsiDNA, a cholesterol form of Dbait, in hematologic malignancies. We show that AsiDNA, enters cells via LDL receptors and activates its molecular target, the DNA dependent protein kinase (DNA-PKcs) in 10 lymphoma and leukemia cell lines (Jurkat-E6.1, MT-4, MOLT-4, 174xCEM.T2, Sup-T1, HuT-78, Raji, IM-9, THP-1 and U-937) and in normal primary human PBMCs, resting or activated T-cells, and CD34+ progenitors. The treatment with AsiDNA induced necrotic and mitotic cell death in most cancer cell lines and had no effect on blood or bone marrow cells, including immune activation, proliferation or differentiation. Sensitivity to AsiDNA was independent of p53 status. Survival to combined treatment with conventional therapies (etoposide, cyclophosphamides, vincristine, or radiotherapy) was analyzed by isobolograms and combination index. AsiDNA synergized with all treatments, except vincristine, without increasing their toxicity to normal blood cells. AsiDNA is a novel, potent, and wide range drug with the potential to specifically increase DNA damaging treatment toxicity in tumor without adding toxicity in normal hematologic cells or inducing immune dysregulation.
Biau J., Chautard E., De Koning L., Court F., Pereira B., Verrelle P., Dutreix M. (2017 Jul 1)

Predictive biomarkers of resistance to hypofractionated radiotherapy in high grade glioma

*Radiation Oncology* : 12 : 123 : [DOI: 10.1186/s13014-017-0858-0]

**Summary**

**Background:** Radiotherapy plays a major role in the management of high grade glioma. However, the radioresistance of glioma cells limits its efficiency and drives recurrence inside the irradiated tumor volume leading to poor outcome for patients. Stereotactic hypofractionated radiotherapy is one option for recurrent high grade gliomas. Optimization of hypofractionated radiotherapy with new radiosensitizing agents requires the identification of robust druggable targets involved in radioresistance.

**Methods:** We generated 11 xenografted glioma models: 6 were derived from cell lines (1 WHO grade III and 5 grade IV) and 5 were patient derived xenografts (2 WHO grade III and 3 grade IV). Xenografts were treated by hypofractionated radiotherapy (6x5Gy). We searched for 89 biomarkers of radioresistance (39 total proteins, 26 phosphoproteins and 24 ratios of phosphoproteins on total proteins) using Reverse Phase Protein Array.

**Results:** Both type of xenografted models showed equivalent spectrum of sensitivity and profile of response to hypofractionated radiotherapy. We report that Phospho-EGFR/EGFR, Phospho-Chk1/Chk1 and VCP were associated to resistance to hypofractionated radiotherapy.

**Conclusions:** Several compounds targeting EGFR or CHK1 are already in clinical use and combining them with stereotactic hypofractionated radiotherapy for recurrent high grade gliomas might be of particular interest.

Pierre Montay-Gruel, Kristoffer Petersson, Maud Jaccard, Gaël Boivin, Jean-François Germond, Benoit Petit, Raphaël Doenlen, Vincent Favaudon, François Bochud, Claude Bailat, Jean Bourhis, Marie-Catherine Vozenin (2017 May 27)

Irradiation in a flash: Unique sparing of memory in mice after whole brain irradiation with dose rates above 100Gy/s.

*Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* : 365-369 : [DOI: S0167-8140(17)30365-1]

**Summary**

This study shows for the first time that normal brain tissue toxicities after WBI can be reduced with increased dose rate. Spatial memory is preserved after WBI with mean dose rates above 100Gy/s, whereas 10Gy WBI at a conventional radiotherapy dose rate (0.1Gy/s) totally impairs spatial memory.
Charles Fouillade, Vincent Favaudon, Marie-Catherine Vozenin, Paul-Henri Romeo, Jean Bourhis, Pierre Verrelle, Patrick Devauchelle, Annalisa Patriarca, Sophie Heinrich, Alejandro Mazal, Marie Dutreix (2017 Mar 12)

[Hopes of high dose-rate radiotherapy].
Bulletin du cancer : DOI : S0007-4551(17)30031-0

Summary

In this review, we present the synthesis of the newly acquired knowledge concerning high dose-rate irradiations and the hopes that these new radiotherapy modalities give rise to. The results were presented at a recent symposium on the subject.

Marie Dutreix, Michel Marty (2017 Jan 15)

[La Société Française du Cancer is moving forward].

Summary

Year of publication 2016

Julian Biau, Emmanuel Chautard, Frank Court, Bruno Pereira, Pierre Verrelle, Flavien Devun, Leanne De Koning, Marie Dutreix (2016 Aug 29)

Global Conservation of Protein Status between Cell Lines and Xenografts.
Translational oncology : 313-321 : DOI : S1936-5233(16)30044-4

Summary

Common preclinical models for testing anticancer treatment include cultured human tumor cell lines in monolayer, and xenografts derived from these cell lines in immunodeficient mice. Our goal was to determine how similar the xenografts are compared with their original cell line and to determine whether it is possible to predict the stability of a xenograft model beforehand. We studied a selection of 89 protein markers of interest in 14 human cell cultures and respective subcutaneous xenografts using the reverse-phase protein array technology. We specifically focused on proteins and posttranslational modifications involved in DNA repair, PI3K pathway, apoptosis, tyrosine kinase signaling, stress, cell cycle, MAPK/ERK signaling, SAPK/JNK signaling, NFκB signaling, and adhesion/cytoskeleton. Using hierarchical clustering, most cell culture-xenograft pairs cluster together, suggesting a global conservation of protein signature. Particularly, Akt, NFκB, EGFR, and Vimentin showed very stable protein expression and phosphorylation levels highlighting that 4 of 10 pathways were highly correlated whatever the model. Other proteins were heterogeneously conserved depending on the cell line. Finally, cell line models with low Akt pathway activation and low levels of Vimentin gave rise to more reliable xenograft models. These results may be useful for the extrapolation of cell culture experiments to in vivo models in novel targeted drug
discovery.


**Drug Driven Synthetic Lethality: bypassing tumor cell genetics with a combination of Dbait and PARP inhibitors.**


**Summary**

Cancer treatments using tumor defects in DNA repair pathways have shown promising results but are restricted to small subpopulations of patients. The most advanced drugs in this field are Poly(ADP-Ribose) Polymerase (PARP) inhibitors (PARPi), which trigger synthetic lethality in tumors with Homologous Recombination (HR) deficiency. Using AsiDNA, an inhibitor of HR and Non Homologous End Joining, together with PARPi should allow bypassing the genetic restriction for PARPi efficacy.

Marie Dutreix, Michel Marty (2016 Aug 7)

[Not Available].


**Summary**

Pierre-Marie Girard, Atousa Arbabian, Michel Fleury, Gérard Bauville, Vincent Puech, Marie Dutreix, João Santos Sousa (2016 Jul 2)

**Synergistic Effect of H2O2 and NO2 in Cell Death Induced by Cold Atmospheric He Plasma.**

*Scientific reports* : 29098 : DOI : 10.1038/srep29098

**Summary**

Cold atmospheric pressure plasmas (CAPPs) have emerged over the last decade as a new promising therapy to fight cancer. CAPPs’ antitumor activity is primarily due to the delivery of reactive oxygen and nitrogen species (RONS), but the precise determination of the constituents linked to this anticancer process remains to be done. In the present study, using a micro-plasma jet produced in helium (He), we demonstrate that the concentration of H2O2, NO2(-) and NO3(-) can fully account for the majority of RONS produced in plasma-activated buffer. The role of these species on the viability of normal and tumour cell lines was investigated. Although the degree of sensitivity to H2O2 is cell-type dependent, we show that H2O2 alone cannot account for the toxicity of He plasma. Indeed, NO2(-), but not NO3(-...
acts in synergy with H2O2 to enhance cell death in normal and tumour cell lines to a level similar to that observed after plasma treatment. Our findings suggest that the efficiency of plasma treatment strongly depends on the combination of H2O2 and NO2(-) in determined concentrations. We also show that the interaction of the He plasma jet with the ambient air is required to generate NO2(-) and NO3(-) in solution.


Minor changes in the macrocyclic ligands but major consequences on the efficiency of gold nanoparticles designed for radiosensitization. Nanoscale : 12054-65 : DOI : 10.1039/c6nr01228k

Summary

Many studies have been devoted to adapting the design of gold nanoparticles to efficiently exploit their promising capability to enhance the effects of radiotherapy. In particular, the addition of magnetic resonance imaging modality constitutes an attractive strategy for enhancing the selectivity of radiotherapy since it allows the determination of the most suited delay between the injection of nanoparticles and irradiation. This requires the functionalization of the gold core by an organic shell composed of thiolated gadolinium chelates. The risk of nephrogenic systemic fibrosis induced by the release of gadolinium ions should encourage the use of macrocyclic chelators which form highly stable and inert complexes with gadolinium ions. In this context, three types of gold nanoparticles (Au@DTDOTA, Au@TADOTA and Au@TADOTAGA) combining MRI, nuclear imaging and radiosensitization have been developed with different macrocyclic ligands anchored onto the gold cores. Despite similarities in size and organic shell composition, the distribution of gadolinium chelate-coated gold nanoparticles (Au@TADOTA-Gd and Au@TADOTAGA-Gd) in the tumor zone is clearly different. As a result, the intravenous injection of Au@TADOTAGA-Gd prior to the irradiation of 9L gliosarcoma bearing rats leads to the highest increase in lifespan whereas the radiophysical effects of Au@TADOTAGA-Gd and Au@TADOTA-Gd are very similar.


Summary

DT01 is a DNA-repair inhibitor preventing recruitment of DNA-repair enzymes at damage
sites. Safety, pharmacokinetics and preliminary efficacy through intratumoural and peritumoural injections of DT01 were evaluated in combination with radiotherapy in a first-in-human phase I trial in patients with unresectable skin metastases from melanoma.


**Chronic oxidative stress promotes H2AX protein degradation and enhances chemosensitivity in breast cancer patients.**

*EMBO molecular medicine* : 527-49 : DOI: 10.15252/emmm.201505891

**Summary**

Anti-cancer drugs often increase reactive oxygen species (ROS) and cause DNA damage. Here, we highlight a new cross talk between chronic oxidative stress and the histone variant H2AX, a key player in DNA repair. We observe that persistent accumulation of ROS, due to a deficient JunD-/Nrf2-antioxidant response, reduces H2AX protein levels. This effect is mediated by an enhanced interaction of H2AX with the E3 ubiquitin ligase RNF168, which is associated with H2AX poly-ubiquitination and promotes its degradation by the proteasome. ROS-mediated H2AX decrease plays a crucial role in chemosensitivity. Indeed, cycles of chemotherapy that sustainably increase ROS reduce H2AX protein levels in Triple-Negative breast cancer (TNBC) patients. H2AX decrease by such treatment is associated with an impaired NRF2-antioxidant response and is indicative of the therapeutic efficiency and survival of TNBC patients. Thus, our data describe a novel ROS-mediated regulation of H2AX turnover, which provides new insights into genetic instability and treatment efficacy in TNBC patients.

Julian Biau, Flavien Devun, Pierre Verrelle, Marie Dutreix (2016 Feb 27)

**Dbait: An innovative concept to inhibit DNA repair and treat cancer.**

*Bulletin du cancer* : 227-35 : DOI: 10.1016/j.bulcan.2016.01.007

**Summary**

The ability of cancer cells to recognize damage and initiate DNA repair is an important mechanism for therapeutic resistance. The use of inhibitors of DNA damage repair or signaling pathways appears to provide a unique opportunity for targeting genetic differences between tumor and normal cells. In this review, we firstly describe the main DNA lesions induced by the different treatments and the pathways involved in their repair. Then we review the mechanism of action and applications of an innovative DNA repair inhibitor: Dbait (and its clinical form DT01). Dbait/DT01 consists of 32bp deoxyribonucleotides forming an intramolecular DNA double helix that mimics DNA lesions. They act as a bait for DNA damage signaling enzymes, the polyadenyl-ribose polymerase (PARP), and the DNA-dependent kinase (DNA-PK), inducing a “false” DNA damage signal and ultimately inhibiting recruitment at the damage site of many proteins involved in double-strand break and single-strand break repair.
pathways. Preclinical studies have demonstrated the capacity of Dbait/DT01 to improve the efficiency of (i) chemotherapy in colorectal cancer or hepatocellular carcinoma models, (ii) radiofrequency ablative in colorectal cancer liver metastases models, and (iii) radiotherapy in xenografted mice with head & neck squamous cell carcinoma, glioblastoma and melanoma. Following this good preclinical results, we performed a first-in-human phase 1-2a study evaluating the safety and efficacy of the combination of DT01 with radiotherapy for the treatment of skin metastases of melanoma. Twenty-three patients were included. No dose-limiting toxicity was observed. An objective response was observed in 59% lesions, including 30% complete responses. This first promising clinical efficacy provides future potential interesting clinical development of Dbait/DT01 with various anticancer treatments.