Novel orally available DNA-PK inhibitors for enhancement of radiotherapy

Andrew Minchinton, Alastair Kyle , Jennifer Baker, Judit Banath, Taixiang Wang & Anam Liu — BC Cancer Research Institute, Vancouver, Canada
Steve Arns, Tom Hsieh, Jay Paquette, Fahimeh Shidmoossavee, Jason Tan, Claudio Sturino & Joseph Mancini — adMare Bioinnovations, Montreal & Vancouver, Canada — Simon Osborne, Martin Ambler & Ela Smiljanic-Hurley — LifeArc, Stevenage, UK

Overview

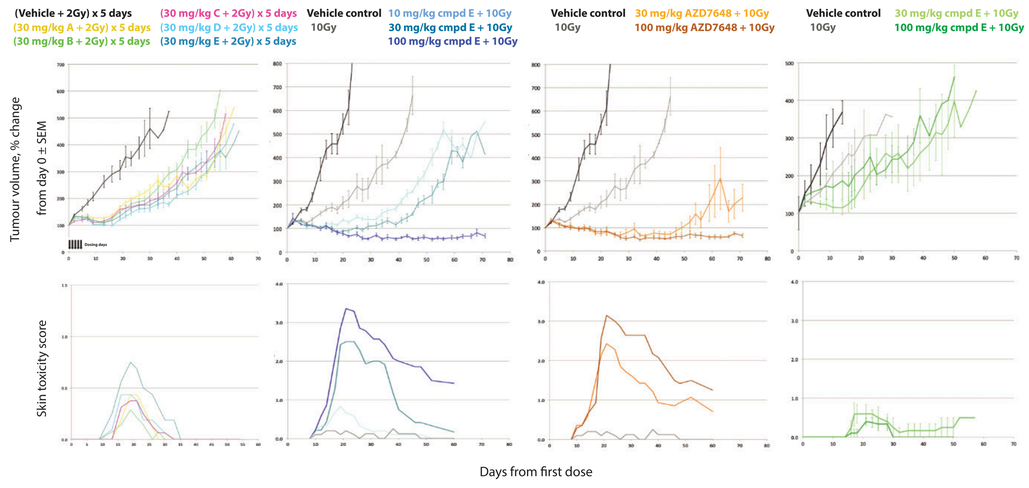
Inhibiting DNA-PK in combination with radiotherapy and chemotherapy shows significant promise and is a highly compelling strategy, with some candidates already in clinical trials. **Here we present our drug development program with novel, orally available, ubiquitously active effector and targeted prodrug DNA-PK inhibitors with excellent potency and *in vivo* activity comparable to competitors.** A shortlist of orally available candidates have been selected from >1000 novel synthesized compounds in two distinct chemical classes. Biochemical, *in vitro* and 3D tissue screens show these compounds with high potency and selectivity, activity throughout the tumour microenvironment, and desirable ADME characteristics. These agents also sensitize cancer models to radiation, in single dose and fractionated regimens *in vivo*. PK/PD and toxicity studies in mice, rats and dogs indicate characteristics suitable for clinical application.

Select data for *effector* candidate shortlist in pre-clinical development

	cmpd A	cmpd B	cmpd C	cmpd D	cmpd E
DNA-PK IC_{50} (nM)	0.33	0.35	0.18	0.17	0.57
DNA-PK MSD FaDu IC_{50} (nM)	20	151	95	68	59
Spheroid IHC HCT116 EC_{50} (nM)	425	892	180	328	250
Selectivity (mTOR/ATM/ATR/PI3Kα, β, δ, γ)	> 657	> 159	> 226	> 764	>1993
Kinase Selectivity (ScanEDGE 97, 1 uM, S(35))	0.044	0.044	0.033	0.033	0.011
Safety Panel (44 targets, 10 uM)	KOP (71%), COX2 (50%)	No hits	COX2 (52%), PDE3A (53%), PDE4D2 (55%)	11 hits > 50 % inhibition	No hits
FW (g/mol)	550	435	531	549	396
clogP	1.7	2.5	2.8	2.7	2.1
TPSA (Å)	124	102	111	101	103
Kinetic Solubility (µM, pH 7.4)	259	> 625	53	290	332
Solubility (µM, PBS / SIF / SGF)	188 / 188 / > 200	> 200 / > 200 / > 200	72 / 59 / > 200	118 / 180 / > 200	> 200 / > 200 / > 200
Microsomal stabilityCl_{int} (µL/min/mg),	< 116 / 440 / < 116 / 188	< 116 / < 116 / < 116 / < 116	< 116 / 194 / < 116 / < 116	121 / < 116 / < 116 / < 116	< 116 / < 116 / < 116 / < 116
% rem. at 60 min.	83 / 8 / 70 / 33	53 / 98 / 89 / 91	66 / 28 / 63 / 66	45 / 94 / 79 / 64	71 / 81 / 98 / 90
Hepatocyte stability. Cl_{int} (µL/min/10^6 cells)	< 8.2 / 12.4 / < 8.2 / < 8.2	< 8.2 / < 8.2 / < 8.2 / < 8.2	< 8.2 / 9.1 / < 8.2 / < 8.2	< 8.2 / < 8.2 / < 8.2 / < 8.2	< 8.2 / < 8.2 / < 8.2 / < 8.2
% rem. at 120 min.	73 / 35 / 84 / 67	76 / 54 / 105 / 82	88 / 50 / 76 / 96	88 / 75 / 85 / 72	79 / 61 / 116 / 109
hERG (IC_{50}, µM)	26	> 30	> 30	6.5	> 30
CYP Inhibition (HLM)	> 100, > 100, 35, 8.7, 0.54,	> 100, > 100, 83, 8.2, 0.34,	> 100, > 100, > 100, > 100,	> 100, > 100, > 100, > 100,	> 100, 9.7, > 100, > 100, >
(IC_{50}, µM: 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4)	4.5, 16	1.9, 7.2	8.2, 9.4, 17	> 100, 3.7, > 100	100, > 100, > 100
PXR Activation (EC_{50}, µM)	> 10	> 10	> 10	8.5 (53 % of rifampicin, 10 µM)	> 10

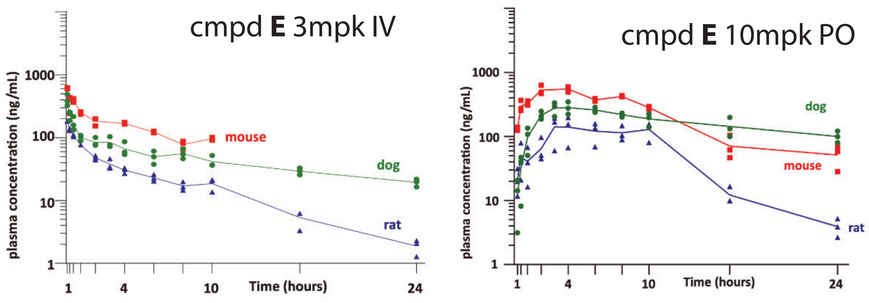
Efficacy in vivo

Novel DNA-PK inhibitors show *in vivo* radiosensitization in FaDu head and neck xenograft models that is comparable with competitors AZD7648 and M3814. Compounds were administered orally in fractionated (administered 1h prior to 2Gy for 5 days) or single dose (administered 1h prior to and again 7h after 10Gy) radiation regimens with radiation delivered to the tumour-bearing leg. Radiation-induced dermatitis on the skin overlaying the tumour was scored by a blinded observer. Onset and intensity of skin damage scores correspond to the dose-sensitive growth delay results.



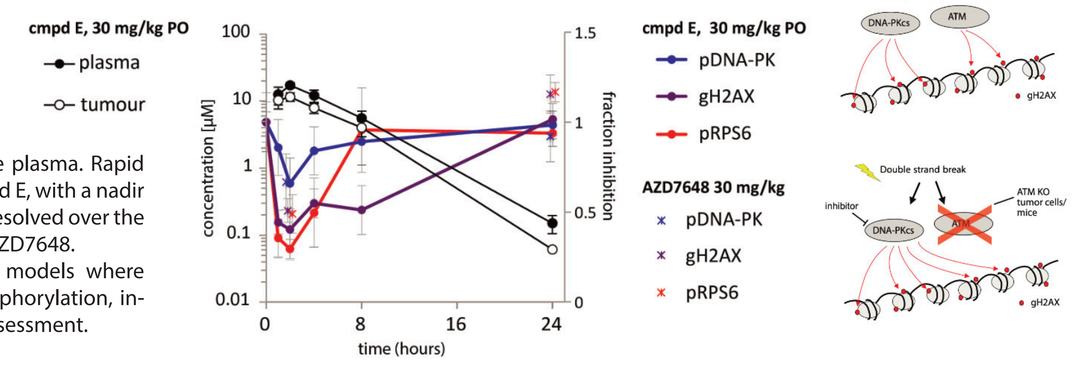
PK mouse / rat / dog

Examples with excellent cross-species pharmacokinetics profiles (cmpd E).



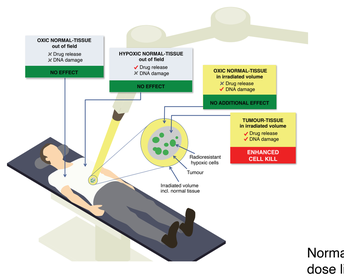
PK/PD inhibtion in mice bearing tool cell line HCT116 ATM^{KO} xenografts

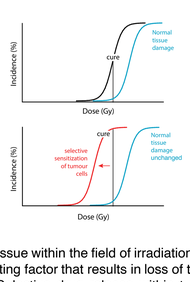
Using immuno-markers of DNA damage we examined DNA repair inhibition induced by effector cmpd E over time, as an example of our shortlisted compounds, and compared it with AZD7648 (overlaid). Tumour concentrations tracked those in the plasma. Rapid inhibition of DNA repair was induced by cmpd E, with a nadir ~2h after PO administration. This inhibition resolved over the following 24h period and is comparable to AZD7648.
This work is performed in ATM^{KO} tumour models where gH2AX is a direct indicator of DNA-PK phosphorylation, increasing the sensitivity of DNA-PK activity assessment.



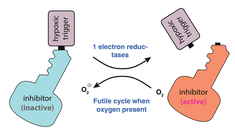
Ongoing *prodrug* development for improved tumour selectivity

Hypoxia activated-prodrug concept: Targeted inhibition of DNA-repair to enhance radiotherapy while preserving therapeutic index

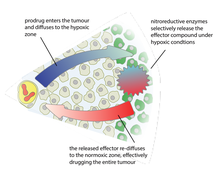




Normal tissue within the field of irradiation is the dose limiting factor that results in loss of tumour control. Selective drug release within tumours will minimize sensitization of normal tissue thereby increasing the therapeutic window.

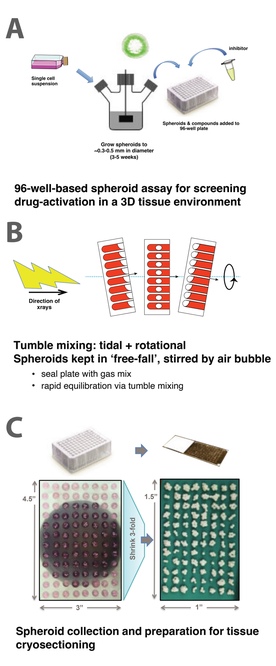


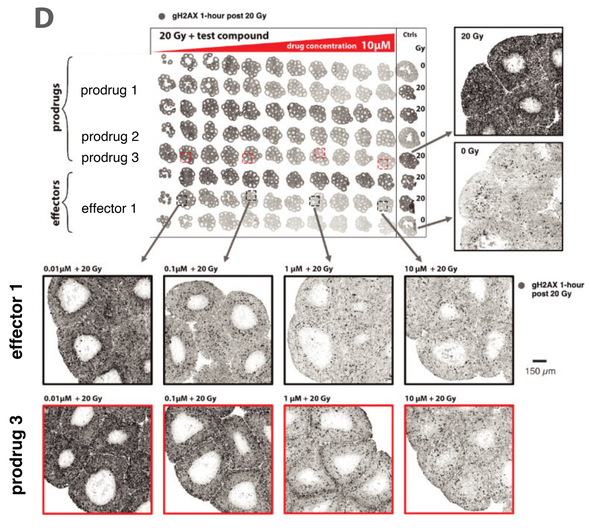
DNA-PK inhibitor activity is blocked by a nitro-heterocyclic trigger moiety in a similar fashion to other hypoxia activated prodrugs (evofosfamide). Under hypoxic conditions found within tumours a 1e enzyme driven reduction occurs which results in release of the active compound.



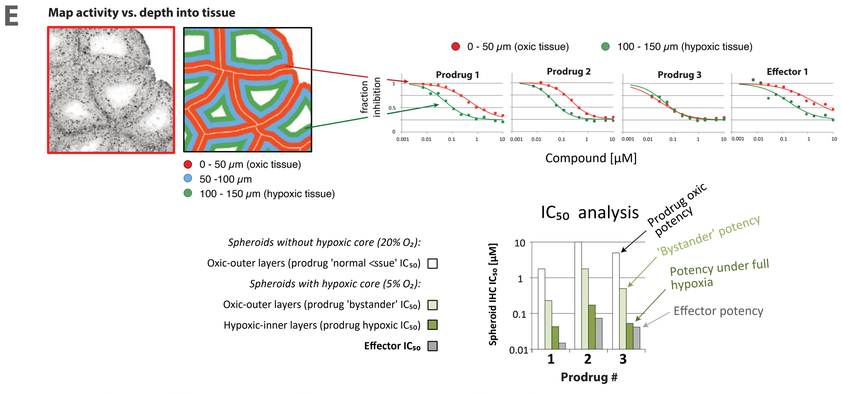
Hypoxic zones within tumours act as an engine to convert prodrug to active form. Once released the effector can then re-diffuse to oxic-zone and inhibit DNA repair throughout the tumour.

3D tissue screening assay for prodrug optimization





Immunostained micro arrays of spheroids (SMA) treated with candidates in combination with radiation. This assay using ATM^{KO} cells effectively models extravascular distribution, a key parameter for the effectiveness of anticancer agents, and can screen for activity specifically in tissues of varying degrees of hypoxia.



IC50s derived from SMAs allow comparison of prodrug potency on inner vs outer spheroid cell layers. Prodrugs are screened for absolute potency in hypoxic inner layers as well as their ability to exert a bystander effect on outer oxygenated layers.

Conclusions

This ongoing drug development program has produced novel, orally available DNA-PK inhibitors. Ubiquitously active, systemic inhibitors are potent with in vivo activity comparable to competitors. Hypoxia-activated prodrugs screening in 3D spheroids show good extravascular distribution with evidence of activity specifically in hypoxic regions.

Radiation sensitization via DNA-PK inhibitors has significant potential for improving cancer control.

Funding for this development project from Canadian Institute of Health Research, The Wellcome Trust, adMare BioInnovations, LIfeArc & BC Cancer