camptothecin. *dss1*⁻ mutant cells also showed wild type sensitivity to UV light and ionizing radiation. The fission yeast cells lacking *dss1*are unusual in that they are both cold sensitive and temperature sensitive for growth. *dss1*⁻ cells that are capable of growth at higher temperature can be readily isolated, and retain enhanced sensitivity to topo II targeting agents. These cells retain cold sensitivity for growth. Our results indicate that *Dss1*has separable functions that are important for DNA repair or growth. Current experiments are examining proteins that interact with Dss1, which may illuminate repair processes that require Brca2 in mammalian cells.

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Preclinical investigation of novel inhibitors of DNA dependent protein kinase

M.A. Batey[1], Y. Zhao[1], H.D. Thomas[1], G.C.M. Smith[2], D.R. Newell[1], N.J. Curtin[1]. [1]*Northern Institute for Cancer Research, Newcastle, UK;* [2]*KuDOS Pharmaceuticals, Cambridge, UK*

The repair of DNA double strand breaks (dsb) is critical for the survival of cells exposed to ionising radiation or chemical agents such as topoisomerase II poisons. In mammalian cells non-homologous end-joining (NHEJ) is the main pathway for repair of DNA dsb in which DNA-dependent protein kinase (DNA-PK) is a major participant. DNA-PK deficient cells are hypersensitive to ionising radiation and some DNA-damaging anticancer drugs, and inhibition of DNA-PK therefore represents a potential strategy for radio- and chemo-sensitization.
The catalytic subunit of DNA-PK (DNA-PKcs) is a member of the phosphatidylinositol (PI) 3-kinase like kinase (PIKK) family of serine/threonine protein kinases. LY294002, a PI 3-kinase inhibitor also inhibits DNA-PKcs, and sensitizes tumour cells to ionizing radiation and dsb-inducing chemotherapeutics. NU7441 is a more potent and specific novel DNA-PK inhibitor (IC50 = 12 nM) developed from LY294002.
The cellular specificity NU7441 for DNA-PKcs was studied in V3 and V3-YAC cells, deficient and proficient in DNA-PKcs respectively. V3 cells were inherently more sensitive to ionising radiation and etoposide (a topoisomerase II poison) than V3-YAC cells and NU7441 increased the radiosensitivity of V3-YAC cells but not of V3 cells. NU7441 also potentiated etoposide cytotoxicity in V3-YAC cells but not V3 cells, confirming that DNA-PKcs is the cellular target of NU7441.
Exposure of the human colon cancer cell lines LoVo and SW620 to 1 μM NU7441 for 16hr did not effect cell survival but enhanced the cytotoxic effects of both etoposide and doxorubicin.

	% Survival		Dose mod[a]	% Survival		Dose mod[a]
	Etoposide (100 nM)	Etoposide + NU7441		Doxorubicin (10 nM)	Doxorubicin + NU7441	
LoVo	68	18	3.7	41	24	1.7
SW620	84	47	1.8	61	36	1.7

[a]Dose modification.

Plasma pharmacokinetic analyses performed following intravenous (i.v), intraperitoneal (i.p.) and oral (p.o.) administration showed 100% i.p. bioavailability and 33% p.o. bioavailability. Following i.p. administration peak plasma levels were 2.4 μg/ml, AUC was 150 μg/ml*min and the $T_{1/2}$ was 50 min. Administration of 10 mg/kg NU7441 i.p. daily for 5 days was well tolerated and did not cause significant weight loss. Tissue distribution studies conducted in SW620 xenograft bearing mice show that NU7441 was well distributed to the tumour and other tissues, where it was retained following clearance from the plasma. Levels of NU7441, commensurate with chemo and radiosensitization in vitro were maintained in tumour tissue for approximately 4hr.
These experiments demonstrate that the cellular effects of NU7441 are specific for DNA-PKcs and that the concentrations required for chemo and radiosensitization in vitro can be achieved in tumour xenografts following i.p. administration of well tolerated doses of NU7441.

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Impact of the DNA repair efficiency in the outcome of sarcoma patients treated with ET-743 (Yondelis)

A. van Oosterom[1], R. Maki[2], M. Taron[3], R. Sciot[1], J.M. Fernandez Sousa-Faro[4], J.C. Tercero[4], J. Jimeno[4], R. Rosell[3]. [1]*UZ Gasthuisberg, Oncology, Leuven, Belgium;* [2]*MSKCC, Oncology, New York, United States;* [3]*Hospital Univ. Germans Trias i Pujol, Oncology, Badalona, Spain;* [4]*PharmaMar, Scientific Development, Madrid, Spain*

ET-743 (trabectedin, Yondelis) induces long lasting objective remissions and tumor control in a subset of patients (pts) with advanced pretreated sarcoma (SA). ET-743 cytotoxicity in experimental models directly correlates with efficient DNA repair. XPD and BRCA1 are involved in transcription-coupled nucleotide excision repair (NER) and in homologous recombination repair, while ERCC1 is involved in global genome NER. On this basis, we performed a retrospective study to correlate the polymorphisms of the XPD (Lys751Gln and Asp312Asn) and ERCC1 (C118T) endonucleases, as well as the mRNA expression levels of ERCC1, XPD and BRCA1 with the pts clinical outcome to ET-743 therapy. Paraffin embedded tumor samples obtained from the pts before treatment were analysed by quantitative RT-PCR in a blind manner by which the investigators were unaware of the clinical data. Fifty-three heavily pretreated pts were included in the study. The overall response (RR) rate in 45 evaluable pts was 11% (5 PRs) and 10 pts (22%; 5 PRs, 1 MR and 4 SD) achieved progression free survival $\geqslant$ 6 months (PFS6). Median survival was 17 months (22 pts still censored). The highest RRs were observed in pts homozygous for wild-type XPD Lys/Lys (20%) and Asp/Asp (19%) as compared to 5 and 7% in the heterozygous, and no responses in pts homozygous for variant genotype Gln/Gln and Asn/Asn. Additionally, pts harbouring high levels of ERCC1 and XPD mRNA expression have higher PFS6 rates of 32% and 25% vs 16% and 18% respectively. However, low levels of BRCA1 mRNA expression appear to increase both the PFS6 (35% vs 6%, [p=0.06]), and the median survival (19 vs 6 months, [p=0.04]) compared to those with high BRCA1 expression levels. Therefore, polymorphisms and expression levels of the DNA repair genes XPD, ERCC1 and BRCA1 may induce differential sensitivity to ET-743 in SA patients. These results merit further validation in a prospective setting in SA and other tumours.

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The ING family tumor suppressor genes enhance nucleotide excision repair

E.I. Campos[1], W.H. Kuo[1], M.Y. Chin[1], D.L. Mitchell[2], G. Li[1]. [1]*University of British Columbia, Medicine, Vancouver, Canada;* [2]*University of Texas, Carcinogenesis, Smithville, USA*

Background: The ING1 (inhibitor of growth 1) tumor suppressor gene plays an important role in cellular stress response to ultraviolet (UV) radiation, such as cell cycle arrest, apoptosis, and DNA repair. Four additional related genes (ING2–5) have recently been identified and shown to possess tumor suppressive functions.
Materials and Methods: A host cell reactivation assay was used to study the DNA repair functions of ING proteins.
Results: We have previously shown that the ING1b gene enhances the repair of UV-induced DNA damage. Furthermore, sequencing of the ING1 gene in human cutaneous melanoma biopsies revealed that mutations of the ING1b gene are detrimental to DNA repair. In addition, we found that treatment with the histone deacetylase inhibitor trichostatin A resulted in an increase in DNA repair efficiency in cells overexpressing mutant ING1b gene to the level equivalent to cells-transfected with wild-type ING1b gene, suggesting that ING1b may activate histone acetylation. Local irradiation and immunofluorescence reveals that p33ING1b, together with the histone acetyltransferase p300, is expressed in the entire nucleus and is not localized to UV-induced lesions, suggesting that p33ING1b may facilitate acetylation of histones 3 and 4 upon UV irradiation, thus act as a chromatin accessibility factor. Moreover, melanoma patients that harbor ING1 mutations in the tumors may be at higher risk to die from the disease within 5 years (50%) compared to patients with no ING1 mutation (18%). We further demonstrate that ING2–5 proteins also enhance nucleotide excision repair of UV-induced DNA lesions.
Conclusion: Taken together, our data indicates that ING genes enhance nucleotide excision repair, which leads to increased genomic stability.

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Expression and prognostic significance of phosphorylated histone H2AX in chronic myelogenous leukemia

J. Cheong[1], H. Jeung[2], J. Kim[2], J. Lee[2], S. Lee[1], J. Hahn[1], Y. Ko[1], Y. Min[1]. [1]*Yonsei University College of Medicine, Department of Internal Medicine, Seoul, Republic of Korea;* [2]*Yonsei University College of Medicine, Clinical Research Center, Seoul, Republic of Korea*

Background: H2AX function is essential for mammalian DNA repair and genomic stability. DNA double-strand breaks cause rapid phosphorylation of the histone H2AX (γ H2AX), which is associated with the recruitment of repair factors to damaged DNA. The progression of chronic myelogenous leukemia (CML) from chronic phase toward acute phase is generally accompanied by an increased Bcr-Abl in leukemic cells, with evidence of additional genetic and chromosomal abnormalities, suggesting a genetic instability in Ph1 cells. We hypothesized that the H2AX could also play a role in this process.