# Attacking bladder cancer

## Turning off the stress defence system in bladder cancer cells during chemotherapy provides a promising new therapeutic approach as scientists at the Norwegian University of Science and Technology have discovered

Professor Marit Otterlei and colleagues at the Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology (NTNU) have identified a new therapeutic intervention point with high relevance for the treatment of Non-Muscle Invasive Bladder Cancers.

NMIBC are cancers where the tumours are confined to the bladder mucosa, without penetrating the surrounding muscle layer. About 70% of the patients diagnosed with bladder cancer have NMIBC; these are usually treated by transurethral resection to remove all visible cancerous tissue, followed by postoperative intravesical instillation of chemotherapy and/or immunotherapy.[1] An important challenge in the treatment of NMIBC patients is the risk of recurrence, reaching 61-78% after one and five years respectively, in high-risk patients.[2,3] Consequently, all patients need to be closely monitored by periodical cystoscopy examinations creating both a burden for patients and high costs for the healthcare systems; thus, there is an urgent need for improvement of NMIBC management.

During studies in DNA repair, Otterlei and colleagues discovered that direct repair of methylated DNA by the human AlkB homolog 2 was coupled to replication.[4] Many of the proteins coupled to replication directly interact with the replication organizer protein Proliferating Cell Nuclear Antigen (PCNA) via a sequence known as the PIP-box.[5] They found that AlkB homolog 2 interacted with PCNA via a previously unidentified interacting motif that they termed APIM (AlkB PCNA Interacting Motif).[6] PCNA is an essential

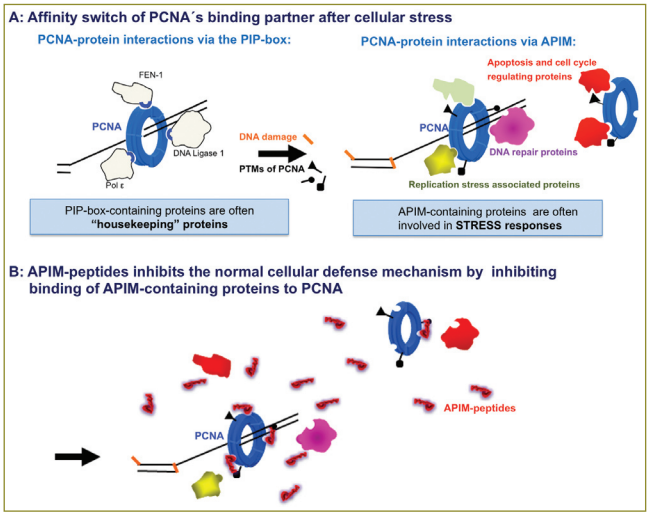
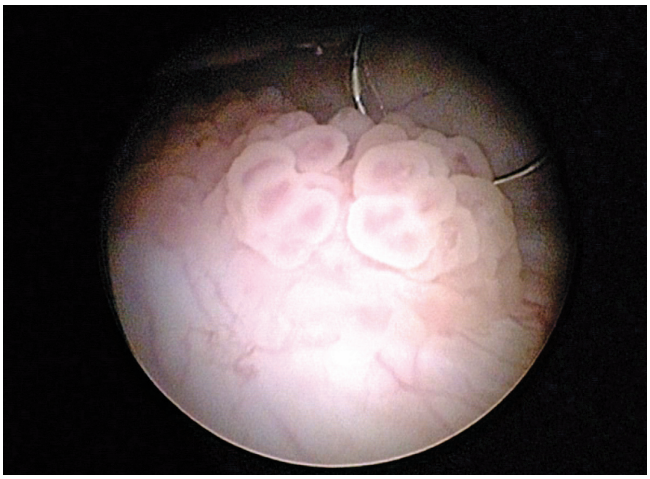



*Fig. 1 Mechanism of action of APIM-peptides*



cellular protein. It is required for the organisation of DNA replication, chromatin remodelling/epigenetics and DNA repair,[7,8] and is implicated in regulation of apoptosis.[9,10]

APIM is found in more than 200 proteins, and interestingly, the majority of these proteins are involved in cellular stress responses. Peptides containing APIM (APIM-peptides) bind to PCNA and increase the efficacy of more than 25 different chemotherapeutics as well as gamma-irradiation, likely by competitive inhibition of interaction between stress defense proteins containing APIM and PCNA. Thus, APIM-peptides impair the cells' defence system against stress, enabling the use of lower doses of drugs to achieve the same efficacy or by enhancing the therapeutic effects of the applied doses (Fig. 1). Based on this approach, engineered cell penetrating APIM-peptides are currently being developed for use in the treatment of NMIBC by NTNU's spinoff company APIM Therapeutics AS (http://www.apimtherapeutics.com/).

PCNA controls key 'housekeeping' processes by interacting with a set of cellular proteins via the PIP-Box (Fig. 1, Panel A, left). Upon cellular stress such as DNA damage, PCNA is post-translationally modified (PTMs) and interacts with APIM-containing proteins (Fig. 1, Panel A, right). Upon targeting PCNA with peptides containing the APIM sequence (APIM-peptide, such as ATX-101), APIM-containing proteins are prohibited from PCNA interaction (Fig. 1, Panel B). This impairs cellular homeostasis and renders cancer cells hypersensitive to death by multiple chemotherapeutic drugs. This has a strong potential for the development of novel combinatorial treatments.

Mitomycin C (MMC) which alkylates and cross-links DNA, is the most commonly used chemotherapeutic for NMIBC treatment.[3,11] Interstrand crosslinks (ICLs) introduced by MMC are highly cytotoxic DNA lesions that involve both DNA strands and result in arrest of both replication and transcription in cells. APIM-peptides interfere with repair mechanisms repairing ICLs and are thus good candidates to increase the cytotoxic effects of of ICL-inducing drugs such as MMC, cisplatin and melphalan.[6,10]

APIM-peptides inhibit DNA repair[6,12] and induce apoptosis in many different cancer cells.[10] The apoptosis inducing activity is divided into an immediate (in less than 2 hours) cleavage of caspases 3/7, 8 and 9, as well as later responses based upon impaired DNA repair and deregulation of signal transduction pathways important in regulation of proliferation and apoptosis[10] (and unpublished results). Multiple myeloma and bladder cancer cells are particularly sensitive to APIM-peptides; other cancer cells and normal cells are not sensitive.[10] This differential sensitivity of cells to APIM-peptide is most likely dependent on the affinity of the APIM-PCNA binding in these cells. This affinity is regulated by multiple factors dependent on the cellular stress levels, including post-translational modifications of PCNA.[6,7] On the contrary, the other known PCNA interacting sequence, the PIP-box, is found in most of the proteins essential for replication and the PIP-box has strong affinity for PCNA under normal conditions (Fig. 1). This is likely why PIP-box peptides are found to be cytotoxic to cells also in absence of cellular stress.[13]

Exploiting this cancer-specific mechanism of action, ATX-101, the prototype APIM-containing peptide has shown strong *in vivo* anticancer efficacy in five animal cancer models; three xenograft mice models (prostate, multiple myeloma and acute myeloid leukemia) and two orthotopic bladder cancer models in immune competent rats (AY-27 and BBN). In all of these models, they detected no toxicity of the peptide, in strong support of *in vitro* results suggesting low activity in normal cells and a high therapeutic index in cancer tissues.

Future development plans include execution of a battery of safety pharmacology tests with ATX-101 as required by regulatory authorities prior to clinical phase entry. Originally to be tested in NMIBC patients of intermediate risk in a phase I/IIa trial, ATX-101 may deliver clinical proof-of-concept within the next two to three years. If proven successful, this programme may hold promise for the development of a new therapeutic approach that could benefit NMIBC patients.


1 P. Kulshreshtha, N. Kannan, R. Bhardwaj, S. Batra, Primary squamous cell carcinoma of the renal parenchyma, Indian J Pathol Microbiol, 55 (2012) 370-371.

2 M. Babjuk, W. Oosterlinck, R. Sylvester, E. Kaasinen, A. Böhle, J. Palou-Redorta, M. Rouprêt, EAU guidelines on non–muscle-invasive urothelial carcinoma of the bladder, the 2011 update, European urology, 59 (2011) 997-1008.

3 M.D. Shelley, M.D. Mason, H. Kynaston, Intravesical therapy for superficial bladder cancer: a systematic review of randomised trials and meta-analyses, Cancer treatment reviews, 36 (2010) 195-205.

4 P.A. Aas, M. Otterlei, P.O. Falnes, C.B. Vagbo, F. Skorpen, M. Akbari, O. Sundheim, M. Bjoras, G. Slupphaug, E. Seeberg, H.E. Krokan, Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA, Nature, 421 (2003) 859-863.

5 E. Warbrick, PCNA binding through a conserved motif, Bioessays, 20 (1998) 195-199.

6 K.M. Gilljam, E. Feyzi, P.A. Aas, M.M. Sousa, R. Muller, C.B. Vagbo, T.C. Catterall, N.B. Liabakk, G. Slupphaug, F. Drablos, H.E. Krokan, M. Otterlei, Identification of a novel, widespread, and functionally important PCNA-binding motif, The Journal of cell biology, 186 (2009) 645-654.

7 N. Mailand, I. Gibbs-Seymour, S. Bekker-Jensen, Regulation of PCNA–protein interactions for genome stability, Nature Reviews Molecular Cell Biology, 14 (2013) 269-282.

8 G.L. Moldovan, B. Pfander, S. Jentsch, PCNA, the maestro of the replication fork, Cell, 129 (2007) 665-679.

9 V. Witko-Sarsat, J. Mocek, D. Bouayad, N. Tamassia, J.A. Ribeil, C. Candalh, N. Davezac, N. Reuter, L. Mouthon, O. Hermine, M. Pederzoli-Ribeil, M.A. Cassatella, Proliferating cell nuclear antigen acts as a cytoplasmic platform controlling human neutrophil survival, The Journal of experimental medicine, 207 (2010) 2631-2645.

10 R. Muller, K. Misund, T. Holien, S. Bachke, K.M. Gilljam, T.K. Vatsveen, T.B. Ro, E. Bellacchio, A. Sundan, M. Otterlei, Targeting proliferating cell nuclear antigen and its protein interactions induces apoptosis in multiple myeloma cells, PloS one, 8 (2013) e70430.

11 M. Tomasz, Mitomycin C: small, fast and deadly (but very selective), Chemistry & biology, 2 (1995) 575-579.

12 K.M. Gilljam, R. Muller, N.B. Liabakk, M. Otterlei, Nucleotide Excision Repair Is Associated with the Replisome and Its Efficiency Depends on a Direct Interaction between XPA and PCNA, PloS one, 7 (2012) e49199.

13 E. Warbrick, A functional analysis of PCNA-binding peptides derived from protein sequence, interaction screening and rational design, Oncogene, 25 (2006) 2850-2859.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