Towards a more efficient ensemble of predictive models for cancer drug development with special focus on metastatic bone disease

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Introduction

Prostate cancer is the second most common form of cancer affecting men worldwide, and it has a high affinity for metastasis to bone. As bone metastases are challenging to prevent and treat, clinically predictive preclinical cancer models are urgently needed to promote drug development.

Aim of the Study

Our aim was to establish an optimal strategy for efficacy testing of drug candidates for prostate cancer using well characterized and validated research models.

Materials and Methods

PC-3, LNCaP and VCaP cells (ATCC, USA) were used in the *in vitro* studies. Two known chemotherapy drugs, an anthracycline antibiotic doxorubicin (Sigma-Aldrich) and a nucleoside analog gemcitabine (Eli Lilly), were tested as reference compounds. The cells were cultured for 5 days and the effects of drugs were studied by measuring proliferation of the cells at days 1, 3 and 5 using a commercial WST-1 proliferation kit (Roche Diagnostics).

PC-3, LNCaP and VCaP cells were inoculated orthotopically into the prostate, or intratibially to the bone marrow cavity of male Balb/c nude or nod-scid mice (n=60, age 4-6 weeks). At the same time with inoculation of androgen sensitive prostate cancer cells, DHT pellets were implanted, if needed. Treatment was started on the following day and continued until the end of the study. Mice were sacrificed 28 days after orthotopical inoculation, examined macroscopically and selected tissue samples (primary tumors, iliac and sacral lymph nodes) were collected for further histomorphometric analysis. In the intratibial models, mice were sacrificed 4-12 weeks after inoculation, x-rayed, examined macroscopically, and selected tissue samples were collected. In all studies, blood samples from saphenous vein were collected during the study and at termination.

To assess changes in bone biomarkers, serum tartrate-resistant acid phosphatase (TRACP) 5b and N-terminal propeptide of type I procollagen (PINP) were analyzed using the MouseTRAP and Rat/mouse PINP ELISA kits, respectively (IDS Ltd, UK). Serum prostate specific antigen (PSA) was analyzed by ELISA kit (R&D Systems, USA).

Orthotopic xenograft models

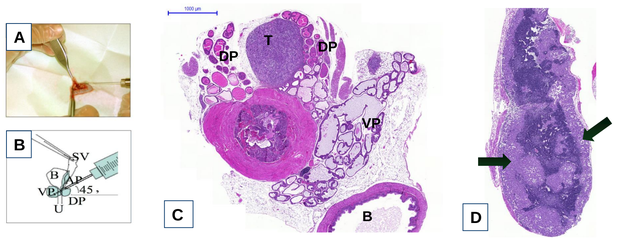

FIGURE 3. A-B) Orthotopic inoculation of prostate cancer cells into mouse ventral prostate through dorsal prostate. **C)** Representative histological image of the orthotopic prostate tumor after inoculation of PC-3 cells. **D)** Orthotopic prostate tumor metastatizes (arrows) to the iliac and sacral lymph nodes. Abbreviations used: SV= seminal vesicle, B= bladder, U= urethra, AP= anterior prostate, VP= ventral prostate, DP= dorsal prostate, T= tumor.

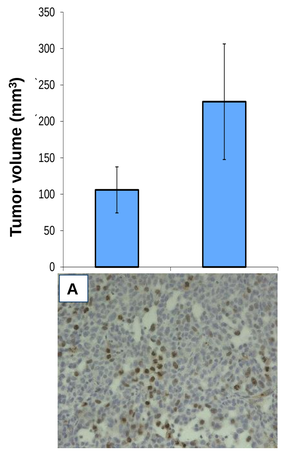

FIGURE 4. Tumor is measured at sacrifice, and tumor volume is calculated according to the height, width and length.

FIGURE 5. Orthotopic tumors were further analyzed by immunohistochemical stainings showing **A)** high proliferation (Ki-67) **B)** apoptotic cells (TUNEL) **C)** number of blood capillaries (CD 34).

Xenograft model of bone metastasis

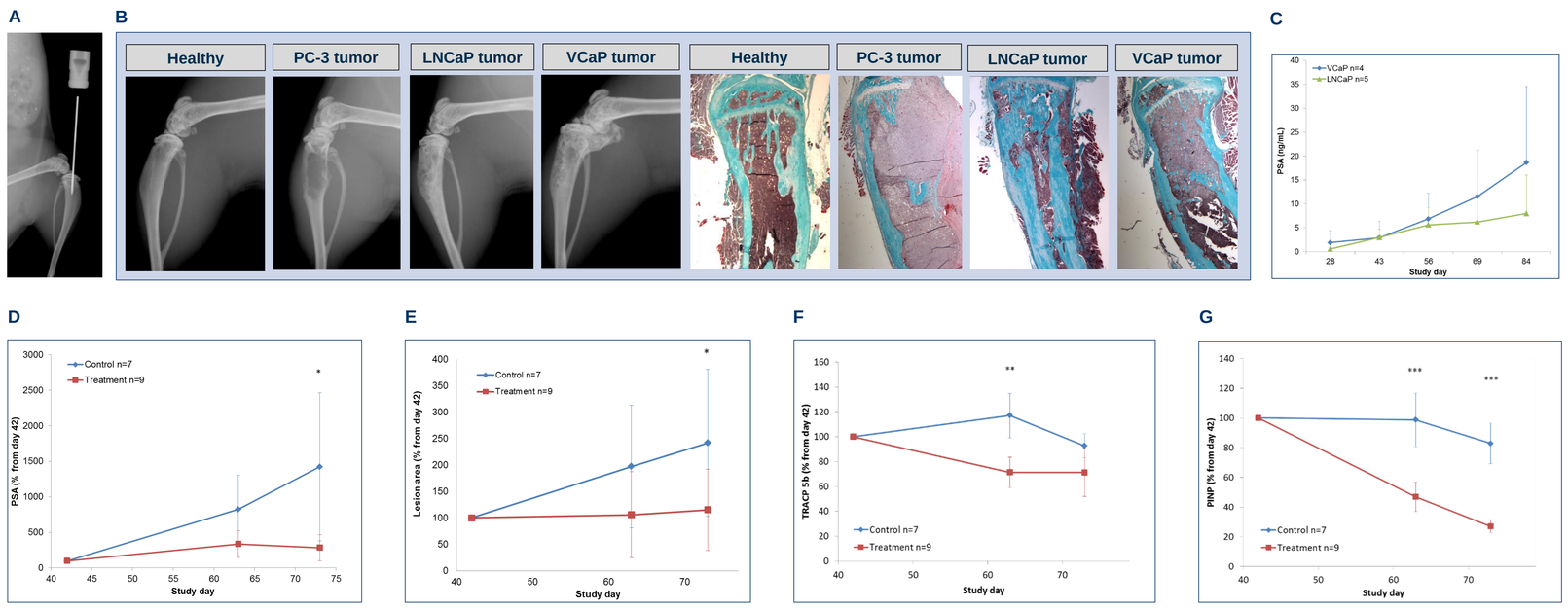

FIGURE 6. A) Site of intratibial inoculation **B)** Representative x-ray and histological images from healthy and tumor-bearing animals with osteolytic or osteoblastic lesions **C)** Comparison of PSA secretion in LNCaP and VCaP intratibial models **D-G)** In the LNCaP intratibial model treatment with an customer compound decreased PSA levels (D), bone lesion area during disease progression (E) and bone biomarkers TRACP 5b (F) and PINP (G).

High throughput screening

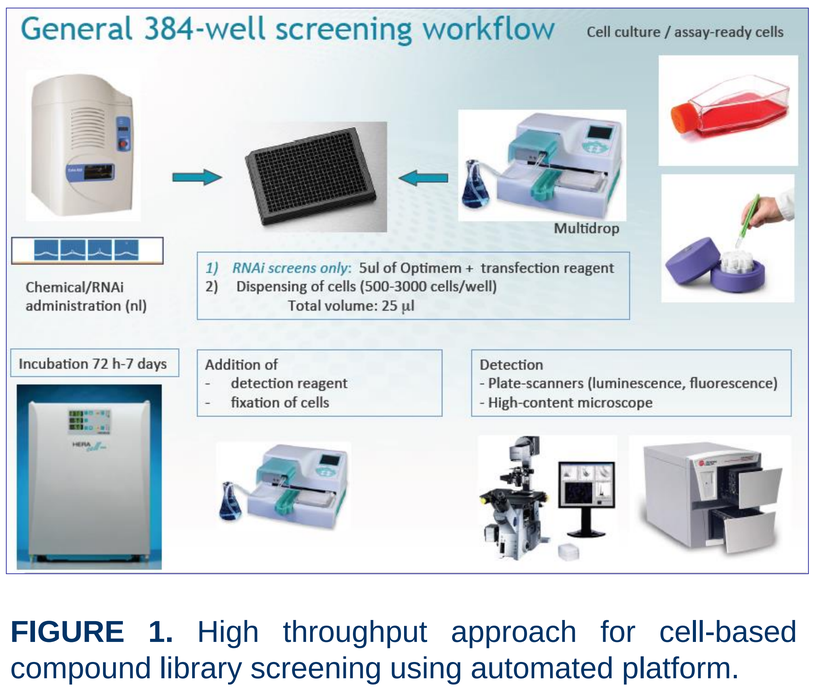

FIGURE 1. High throughput approach for cell-based compound library screening using automated platform.

Efficacy assessment *in vitro*

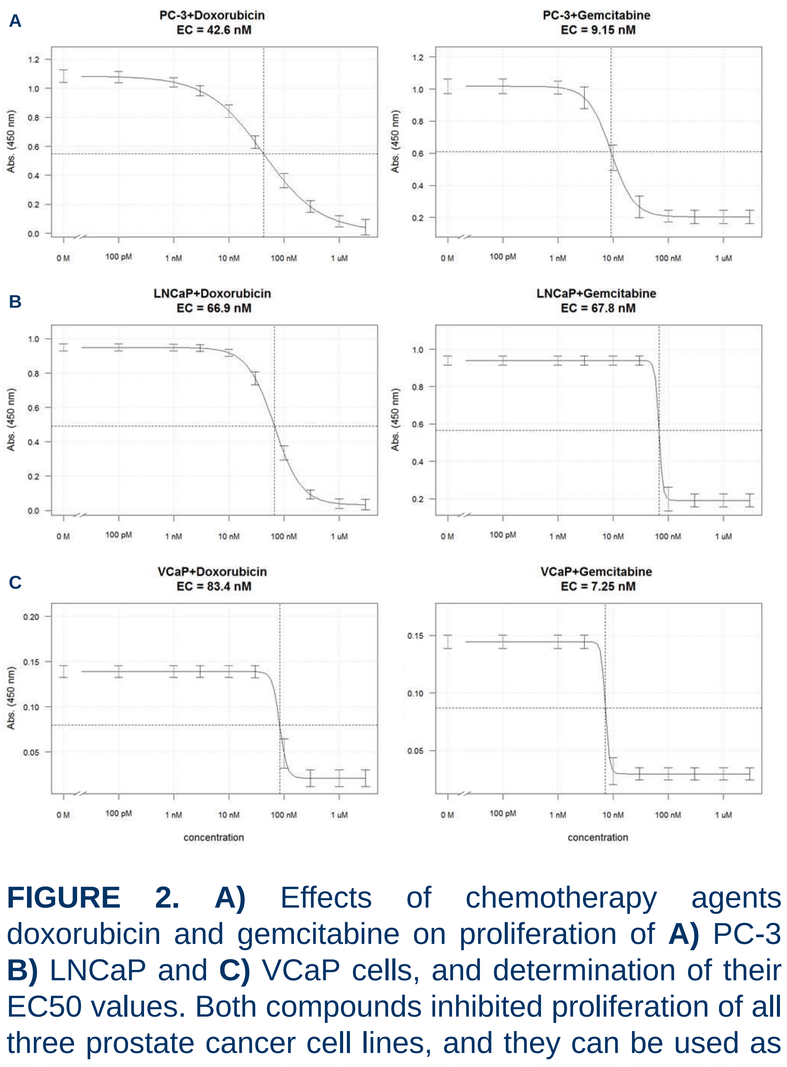

FIGURE 2. A) Effects of chemotherapy agents doxorubicin and gemcitabine on proliferation of **A)** PC-3 **B)** LNCaP and **C)** VCaP cells, and determination of their EC50 values. Both compounds inhibited proliferation of all three prostate cancer cell lines, and they can be used as reference compounds when testing effects of novel drug candidates on proliferation of these cell lines.

Summary

CELL LINE	ROUTE	METASTASIS	READOUT
PC-3	Orthotopic	Lymph node	Tumor volume, lymph node metastasis, histomorphometry
PC-3	Intratibial	Bone (osteolytic)	Radiography, histomorphometry, bone biomarker (TRACP 5b)
LNCaP	Orthotopic	None	Tumor volume, histomorphometry, PSA
LNCaP	Intratibial	Bone (osteoblastic)	Radiography, histomorphometry, PSA, bone biomarkers (TRACP 5b, PINP)
VCaP	Intratibial	Bone (osteoblastic)	Radiography, histomorphometry, PSA, bone biomarkers (TRACP 5b, PINP)

Conclusions

We propose the following strategy for identifying effective prostate cancer drug candidates for clinical studies:

1) High throughput screening of effects of test compounds on proliferation of human prostate cancer cell lines *in vitro*
2) Determining EC50 values for efficacy of selected compounds on proliferation of human prostate cancer cells *in vitro*
3) Xenograft studies to test efficacy of selected compounds in orthotopic and metastasis models using the same human prostate cancer cell lines whose proliferation the compounds inhibited *in vitro*

The strategy has demonstrated to be clinically predictive, and it can be exploited widely in anti-cancer drug development.

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