

## Background

Prostate cancer is the most common neoplasm among men in the United States, second only to lung cancer as a cause of death. Chemotherapy is used to treat advanced stage hormone-refractory prostate cancer, which is defined as progressive disease despite serum castration-level testosterone values. The median time to this progression is about 18 months. For patients who relapse or never respond to hormonal therapy, the addition or substitution of other hormonal agents is unlikely to be of benefit. Second line therapy typically produces responses in 20% of patients at best, and responses are usually brief, with a median survival after progression of about six months. No effective systemic treatment has been established for this condition, although the combination of Mitoxantrone with prednisone and Taxotere has been approved and is generally regarded as standard of care for symptom palliation.

BBR 3576, an aza-anthrapyrazole (5-[2-(dimethylamino)ethylamino]-2-[2-[(2-hydroxyethyl)amino]ethyl]-indazol[4,3-g]isoquinolin-6(2H)-one dihydrochloride salt), synthesized with the aim of providing a safer and more active second generation anthrapyrazole congeners on solid tumors, demonstrated reduced cardiotoxicity compared to doxorubicin (DX) and Mitoxantrone (Mitox), and high antitumor activity in preclinical studies with several human tumor xenografts. Efficacy and safety of the compound was also investigated in several hormone refractory prostate cancer (HRPC) xenograft models, and in the autochthonous Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP). In the latter model, 100% of mice show tumor progression from prostatic intraepithelial neoplasia to invasive carcinoma metastatic to lymph nodes, lung, liver and bone over 12-16 weeks with a median survival of 36 weeks.

## Pathological progression of prostate cancer in the TRAMP model.



(Figure A: from R. Ghignoli et al. Prostate Cancer and Prostatic Diseases (1999) 2, 70-75.

55 week old C57BL/6 mouse: normal prostate and seminal vesicle size.  
 B) 14 week old TRAMP C57BL/6, normal prostate and seminal vesicle size.  
 C) 37 week old TRAMP C57BL/6; marked enlargement of seminal vesicles secondary to invasive, obstructing prostate tumor.  
 D) 52 week old TRAMP C57BL/6; tumor demonstrates a characteristic more spherical, highly vascular prostate carcinoma which ruptures the bladder and erodes with relatively spared normal seminal vesicles.

## Materials and Methods

## Xenograft tumor models

BBR 3576 was evaluated in comparison with standards doxorubicin (DX), Mitoxantrone (Mitox), Taxol, Taxotere and 5-fluorouracil in several human xenograft tumor models: MX-1 breast ca., A2780 ovarian ca., A549, LX-1 lung ca., CX-1, HCT116 colon ca., Hs746T gastric ca., Panc-1 pancreatic ca. and DU-145, JCA-1, PC-3 prostate ca.

The human tumors were subcutaneously transplanted into female athymic (nu/nu) mice, and treatment started when tumors reached an average weight of 100-150 mm<sup>3</sup>. Compounds were administered intravenously once a week for 3 weeks (q7d,3w injections). During the studies the following parameters were monitored: primary tumor growth, mouse body weight, clinical signs and drug related mortality.  
**Antitumor activity parameters:** RTW% = Relative Tumor Weight Inhibition %: 100-(mean tumor weight of treated/mean tumor weight of control or vehicle group) x 100 evaluated 7 days after last treatment.  
**TOX** = Number of toxic deaths/Total number of treated mice.

## TRAMP (transgenic adenocarcinoma of the mouse prostate) mice

The TRAMP (6BTTRAMP) model was generated in a C57BL/6 background utilizing a transgene consisting of a 7426/26 kb fragment of the rat probasin (rPB) gene directing the tissue-specific expression of simian virus 40 (SV40) early genes (T1 antigens; Tag) to the mouse prostate epithelium to effectively abrogate the activity of the p53 and Retinoblastoma (Rb) tumor suppressors in these cells.

In 100% of cases in this mouse model, prostate ca. progresses from prostatic intraepithelial neoplasia to histological cancer to carcinoma metastatic to lymph nodes, lung, liver and bone over 12-16 weeks, with a median survival of 36 weeks.

## Antitumor activity parameters:

Median Survival Time (weeks): Median number of weeks mice survived after tumor transplantation.

T/C: Median survival time of treated mice/median survival time of controls x 100.

Tumor free animals: number of mice with no signs of tumor mass at gross autopsy evaluation.

Statistical analysis was performed using one-way ANOVA Bonferroni: \*p<0.05; \*\*p<0.01; \*\*\*p<0.001

## Cardiotoxicity

Two parameters of myocardial damage were histologically evaluated by light microscopy:

Semi-thin sections of the whole heart, formalin fixed, embedded in resin and stained by H.E. or Toluidine blue methods.

Semiquantitative score of myocytes lesions. The product of severity degree (1 or 2) multiplied by extension degree (from 0 to 5) gives a total cardiotoxic score (T.C.S.) from 0 to 10 for each animal. From the T.C.S. of all group animals, the M.T.S. (mean total score) for each group was calculated.

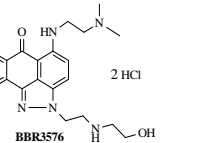
Morphologic evaluation of cardiac lesions Severity: 1= microvacuolation, cellular or interstitial oedema; 2 = same as 1 plus macrovacuolation or amyloid, necrosis, fibrosis, thrombi.

Extension: 0 = no lesion; 0.5 = fewer than 10 single altered myocardiocytes (AM); 1 = scattered single AM; 2 = scattered small groups of AM; 3 = widely spread small groups of AM; 4 = confluent groups of AM; 5 = most cells

## Chemical structure

**Chemical Name**  
 5-[2-(dimethylamino)ethylamino]-2-[2-[(2-hydroxyethyl)amino]ethyl]-indazol[4,3-g]isoquinolin-6(2H)-one dihydrochloride salt

**Chemical Structure:**



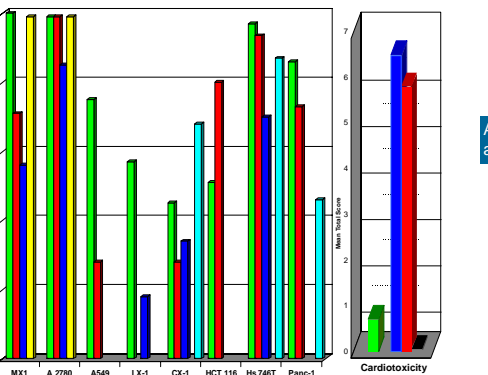
**Molecular Formula:**  
 C<sub>21</sub>H<sub>28</sub>N<sub>6</sub>O<sub>2</sub>Cl<sub>2</sub>

**Molecular Weight:**  
 467.41

**Lab Code:**  
 BBR 3576

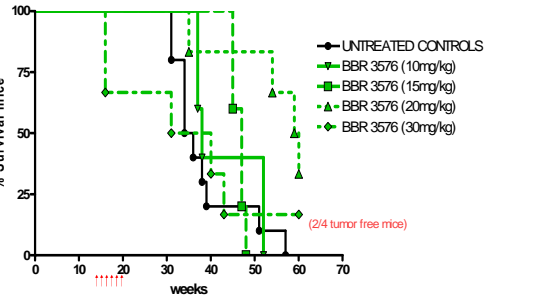
**Description:**  
 orange crystalline powder

Antitumor activity profile of BBR 3576 in comparison with several standards against human xenografts tumors and cardiotoxicity evaluation in comparison with Mitoxantrone (MITOX) and Doxorubicin (DX).



**Optimal doses:**  
 MX-1 Human Mammary Ca.  
 A2780 Human Ovary Ca.  
 A549 Human Lung Ca.  
 LX-1 Human Lung Ca.  
 CX-1 Human Colon Adenoca.  
 HCT116 Human Colon Ca.  
 Hs747 Human Stomach Ca.  
 Panc-1 Human Pancreas Ca.

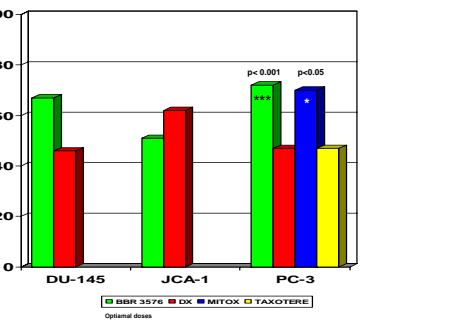
Antitumor activity of BBR 3576 on TRAMP mice following iv weekly schedule treatment (14,15,16,18,19,20 weeks)



BBR 3576 Dose mg/kg	Median survival time (weeks)	T/C%	TOX
controls	35	100	0/10
10	38	109	0/6
15	47	134	0/5
20	59.5**	170	0/6
30	35.5	100*	2/6

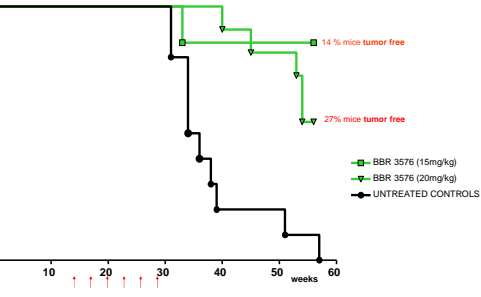
Logrank Test: \* p<0.05 \*\* p<0.01 \*\*\* p<0.001

Antitumor activity of BBR 3576 against human xenografts prostate adenocarcinoma models (s.c./i.v. q7dX3)



Statistical analysis was performed using one-way ANOVA Bonferroni: \*p<0.05; \*\*p<0.01; \*\*\*p<0.001 or unpaired t-test

Antitumor activity of BBR 3576 on TRAMP mice following iv treatment every three weeks for 6 times (14,17,20,23,26,29 weeks)



BBR 3576 Dose mg/kg	# of mice	Median survival time (weeks)	T/C%	TOX	Tumor free at the end of the exp 56' week ( gross autopsy)
controls	10	35	100	0/10	
15	7	>56**	>160	0/7	14% mice tumor free
20	11	>56***	>160	0/11	27% mice tumor free

Logrank Test: \* p<0.05 \*\* p<0.01 \*\*\* p<0.001

## Results and Conclusions

BBR 3576 showed antitumor activity against several xenograft models and also demonstrated less cardiotoxicity than Mitoxantrone and doxorubicin.

## Xenograft HRPC model

BBR 3576 demonstrated equal or superior antitumor activity compared to standard tested agents. In particular against PC-3), antitumor efficacy was significantly superior to that of taxotere and doxorubicin and comparable to that of Mitoxantrone.

## TRAMP Mice model

After weekly iv administration, BBR 3576 showed a dose dependent activity that significantly prolonged the survival of mice compared to untreated animals. Following iv treatment every three weeks X 6 at the dose of 20 mg/kg, 54% of animals survived longer than 56 weeks, and 27% of the animals remained tumor free.

## Conclusions

BBR 3576 showed antitumor activity in a wide spectrum of preclinical tumor models, including human xenograft and transgenic prostate carcinomas, thus supporting further clinical trials in HRPC patients.