Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Thomas Jefferson University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Theodore F. Taraschi, Ph.D.

4. **Grant Contact Person's Telephone Number:** 215-955-3900

5. **Grant SAP Number:** 4100047652

6. **Project Number and Title of Research Project:** 3 – Stat5 and ErbB2 in Prostate Cancer

7. **Start and End Date of Research Project:** 1/1/2009 – 06/30/2012

8. **Name of Principal Investigator for the Research Project:** Marja Nevalainen, MD, Ph.D**.**

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ $461,594.82

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Nevalainen	Assoc Professor	15% Yr 1; 35% Yr 2	$81,166.53
Liao	Research Assoc	100% Yr 1,2; 25% Yr 3	$87,509.49
Dagvadorj	Instructor	60% Yr 1,2; 10% Yr 3	$42,647.33
Ellsworth	Research Tech A	100% Yr 2	$14,308.22
Lutz	Research Assistant B	100% Yr 1,2	$36,545.90

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Elyse Ellsworth	Research Assistant	10

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert “not funded” in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Stat5 in Prostate Cancer Progression	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	July 2011	$1,800,000	$1,350,000

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

NCI, NIH RO1 Stat5 Signaling in Promotion of Prostate Cancer Metastases

12. Future of Research Project. What are the future plans for this research project?

Eight to ten articles to be published, further grant funding to be applied.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				1
Female			1	
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	1
Unknown				
Total			**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian				1
Other				
Unknown				
Total			**1**	**1**

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes__X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    This health research project enabled training of one post-doctoral fellow and one pre-doctoral PhD-student which is the mission of the institution. Also, this health research project led to RO1 grant funding which brings indirect costs to the institution and thereby improves the infrastructure of the institution. Moreover, the project has supported research collaboration with three universities in Europe (Univ. of Turku, Finland, Univ. of Helsinki, Finland and Univ. of Umea, Sweden). Finally, the research project has resulted in two scientific publications (one published and one in submission).

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

    Yes_____X____ No_____

If yes, please describe the collaborations:

Dr. Tuomas Mirtti (Univ. of Turku, Finland, Univ. of Helsinki, Finland) and Dr. Anders Bergh (Univ. of Umea, Sweden).

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X___

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16

months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Goal

In this project, the researcher will test the following central hypothesis: Active transcription factor Stat5a/b mediates resistance of prostate cancer to (neo)adjuvant therapies such as ErbB2 inhibition. Preliminary data demonstrates that active Stat5a/b transcriptionally synergizes with wild-type and mutated androgen receptor (AR) in prostate cancer cells, where Stat5a/b increases transcriptional activity of AR. AR, in turn, increases transcriptional activity of Stat5a/b. Second, we show that epidermal growth factor (EGF) activates Stat5a/b in prostate cancer cells, and, importantly, inhibition of ErbB2 protein expression blocks phosphorylation of Stat5a/b. At the completion of this project it is expected to be established whether activation of Stat5a/b would identify those prostate cancers that respond to ErbB2 inhibition by growth suppression, and whether active Stat5a/b combined with phosphorylated ErbB2 provides a better prognostic indicator of poor clinical outcome than active Stat5a/b alone.

Specific Aims

Aim 1) Determine whether ErbB2 activates Stat5a/b in a set of human prostate cancer cell lines, and whether ErbB2 inhibition leads to apoptosis of human prostate cancer cells and decreases prostate xenograft tumor growth through inhibition of Stat5a/b.

Aim 2) Determine whether Stat5a/b is the mediator of the synergetic interaction between ErbB2 and AR in prostate cancer cells.

Aim 3) Determine whether active Stat5a/b combined with phosphorylated ErbB2 provides a better prognostic indicator of poor clinical outcome than active Stat5a/b alone.

The concept of Stat5a/b as a determinant of therapeutic response of prostate cancer to ErbB2 inhibition is entirely novel. Previous preclinical studies on the role of ErbB2 in the growth of prostate cancer cells have been relatively unsystematic and have yielded conflicting results. The results of clinical trials on ErbB2 inhibitors in prostate cancer have been disappointing. This may be due to clinical trials having focused on advanced prostate cancer only. Second, the trials have not included any selection methods for patients in terms of ErbB2 positivity or activation status of other key growth regulating kinase pathways of prostate cancer. These studies provide the unique opportunity to determine the role of ErbB2 and ErbB2 inhibitors for prostate cancer growth in preclinical models of prostate cancer.

The existing pharmacological therapies for prostate cancer only provide a temporary relief of the symptoms and the cancer growth, while the hormone-refractory cancer develops. In

addition, no effective therapies for metastatic prostate cancer currently exist. Stat5 belongs to the seven-member Stat gene family of transcription factors[2]. There are two highly homologous (98%) isoforms of Stat5, 94-kDa Stat5a and 92-kDa Stat5b, which are encoded by separate genes. Stat5a and Stat5b (hereafter referred to as Stat5a/b) are latent cytoplasmic proteins that act as both cytoplasmic signaling proteins and nuclear transcription factors. Stat5a/b becomes active by phosphorylation of a specific tyrosine residue in the carboxy-terminal domain by a tyrosine kinase. After phosphorylation, Stat5a and 5b homo- or hetero-dimerize and translocate to the nucleus where they bind to specific Stat5a/b response elements of target gene promoters.

Stat5a/b is constitutively active in human prostate cancer but not in normal prostate epithelium[2,5]. This study has shown, in two separate clinical prostate cancer populations, that activation of Stat5a/b is associated with high histological grade of prostate cancer. Moreover, Stat5a/b is in the active state in the majority of hormone-refractory recurrent human prostate cancers. The molecular mechanisms underlying constitutive activation of Stat5a/b in primary and hormone-refractory human prostate cancer are currently unclear. Such mechanisms may involve autocrine Prl-Jak2-pathway as well as the members of the EGF-receptor family. This laboratory discovered that adenoviral expression of a dominant-negative (DN) mutant of Stat5a, blocking both Stat5a and Stat5b, induced massive and rapid apoptotic death of human prostate cancer cells in culture. This DNStat5a mutant lacks the transactivation domain, and thus is able to dimerize and bind to DNA, but is unable to initiate transcription. This finding was later confirmed in the transgenic adenocarcinoma of the **mouse** prostate TRAMP mouse tumor model.

We recently showed that inhibition of Stat5a/b by other methods such as antisense oligo-nucleotides or RNA interference, effectively killed Stat5-positive prostate cancer cells. Importantly, inhibition of Stat5a/b blocked human prostate cancer xenograft tumor growth in nude mice and down-regulated $BclX_L$ and Cyclin-D1 protein levels in prostate cancer cells. Activation of Stat5a/b in primary prostate cancer predicted early disease recurrence, which led us to propose that Stat5a/b may promote castration-resistant growth of prostate cancer..

ErbB1, 2, 3 and 4 are transmembrane tyrosine kinase receptors consisting of an extracellular binding domain, a transmembrane segment and (except for ErbB3) a functional intracellular tyrosine kinase domain. The tyrosine kinase domains are activated by both homo- and heterodimerization, generally induced by ligand binding. In contrast to the three other HER receptors, the extracellular domain of ErbB2 can adopt a fixed conformation resembling a ligand-activated state, permitting it to dimerize in the absence of a ligand. ErbB2 (185 kDa) is the preferred dimerization partner for the other ErbB family members. Despite the fact that ErbB2 gene amplification is uncommon in prostate cancer,ErbB2 protein is over-expressed in prostate cancer, and the molecular mechanisms underlying the protein over-expression are unclear.

In breast cancer, ErbB2 protein overexpression results in constitutive activation of ErbB2 through homodimerization, and HER2 signaling promotes breast cancer growth through the RAS-MAPK and the PI3-kinase-Akt pathways. The spontaneous dimerization partner of ErbB2 or the signaling pathways activated by ErbB2 in human prostate cancer cell lines or in clinical prostate cancers have not yet been identified. Moreover, ErbB2 is known to induce transcriptional activity of androgen receptors (AR) in human prostate cancer cells, but the

signaling pathway that mediates this effect is so far unidentified. The clinical trials of prostate cancer using Tratuzumab (ERbB2/ErbB2-inhibitor) or Pertuzumab (inhibitor of ErbB2 dimerization with other members of the Erb-family) have not indicated objective benefits for tumor growth.It is important to note that the failure of these trials may have been because these clinical trials did not include any methods of patient selection in terms of activation status of key protein kinase signaling pathways (MAPK, Akt, Stat5). This study proposes that Stat5a/b activation in prostate cancer is a prerequisite for a favorable therapeutic response to ErbB2 inhibitors.

This project has demonstrated that Stat5a/b is constitutively active in clinical prostate cancer but not in normal prostate epithelium. Currently, little is known about the molecular mechanisms underlying the constitutive activation of Stat5a/b in prostate cancer cells. Moreover, ErbB2 is known to increase transcriptional activity of AR in prostate cancer cells, but the mediator of this effect has not yet been identified. The clinical trials of prostate cancer treatment involving an epidermal growth factor receptor (EGFR) or ErbB2 inhibitior have yielded disappointing results. It is proposed that EGF activates Stat5a/b through EGFR dimerization with ErbB2 in human prostate cancer, and that Stat5a/b is the mediator of the ErbB2 stimulation of transcriptional activity of AR. Based on this laboratory's preliminary data, it is proposed that Stat5a/b activation in primary prostate cancer is prerequisite for a positive therapeutic response of prostate cancer to ErbB inhibitor compounds.

Methods

Prostate cancer cell lines. DU145, PC-3, CWR22Rv1 and CWR22Pc prostate cancer cells were cultured in RPMI-1640 medium (Biofluids, Rockville, MD) with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Norcross, GA), 2 mM L-glutamine (Biofluids), 5 mM HEPES, pH 7.3 (Biofluids), 50 IU/ml penicillin and 50 µg/ml streptomycin (Biofluids). LNCaP cells were maintained in complete RPMI-1640 medium supplemented with 0.5 nM dihydrotestosterone (DHT).

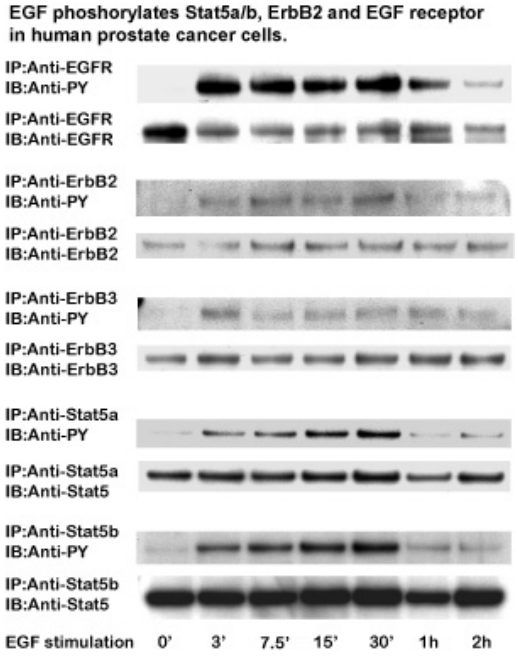
Cell lysis, immunoprecipitation and immunoblotting. For immunoprecipitations of Stat5a/b and EGF receptor family members, PC-3 cells were co-transfected with pPrlR, pGR and pStat5a or pStat5b plasmids, serum-starved for 20 h, and then treated with 1 nM EGF for 16 h. Cells were lysed in lysis buffer [10 mM Tris-HCl (pH 7.5), 5 mM EDTA, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 1% Triton X-100, 1 mM phenylmethylsulphonylfluoride, 5 µg/ml aprotinin, 1 µg/ml pepstatin A, and 2 µg/ml leupeptin], and the protein concentrations of the whole cell lysates were determined by the Bradford method (BioRad Laboratories Inc., Hercules, CA). The cell lysates were immunoprecipitated for 2 h at 4°C with anti-Stat5a, anti-Stat5b pAbs (Advantex Bioreagents, Conroe, TX), or anti-EGF, anti-ErbB2, anti-ErbB1, anti-ErbB3 or anti-ErbB4 pAb (Abcam, La Jolla, CA). Antibodies were captured by incubation for 60 min with protein A-Sepharose beads (Pharmacia Biotech, Piscataway, NJ), and the filters were blotted with anti-Stat5a/b mAb (1:250) (BD Biosciences, San Jose, CA) or with mAb anti-EGFR (1:1000) (BD Biosciences), detected by horseradish peroxidase-conjugated secondary antibodies in conjunction with enhanced chemiluminisence substrate mixture (Amersham, Piscataway, NJ), and exposed to film.

SiRNA transfection**.** DU145 and CWR22Rv1 prostate cancer cells were transfected with the scramble siRNA or siRNAs targeted to human Stat3, Stat5a or Stat5b (Dharmacon, Lafayette, CO, USA) (100 pmol of siRNA) using transfection reagent Lipofectamine 2000 (Invitrogen).

Cell growth and viability assay. The number of alive cells was determined by counting the attached cells using a hemacytometer and trypan blue exclusion. In addition, the cell viability was determined by 3-(4,5 Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay (Promega, Madison, WI). The proportion of viable cells in each treatment group in sextuplicates was determined by tetrazolium conversion to its formazon dye (Promega) at 490 nm (POLARstar OPTIMA, BMG Labtech).

EGF-stimulation of CWR22Rv prostate cancer cells induced activation of Stat5a/b, ErbB2 and EGFR (Fig.1). The cells were starved overnight before stimulation with EGF (17 nM) for indicated periods of time. Stat5a and Stat5b were immunoprecipitated and blotted with anti-phosphoStat5a/b antibody, while activation of EGFR-family members was detected from whole cell lysates.

Fig.1.



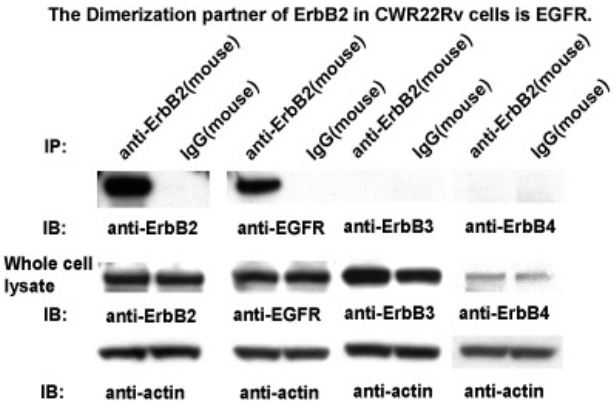


Fig.2.A.

Fig.2.B.

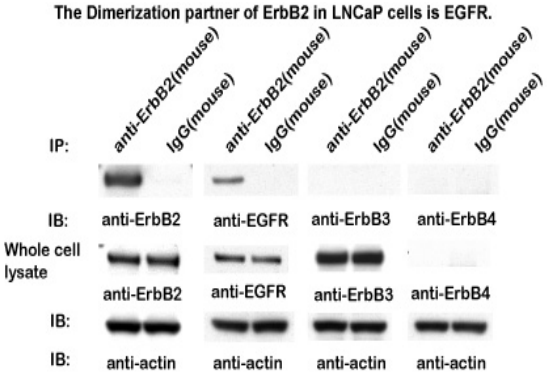
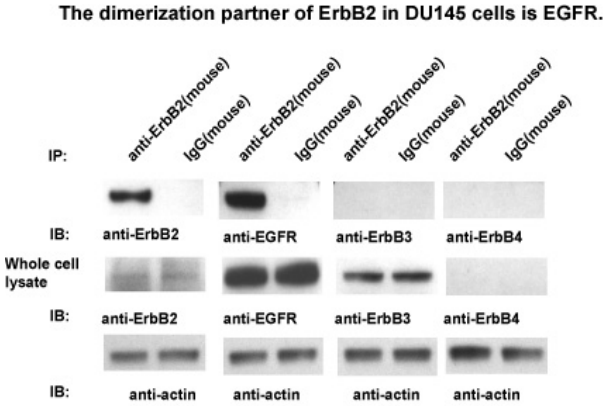
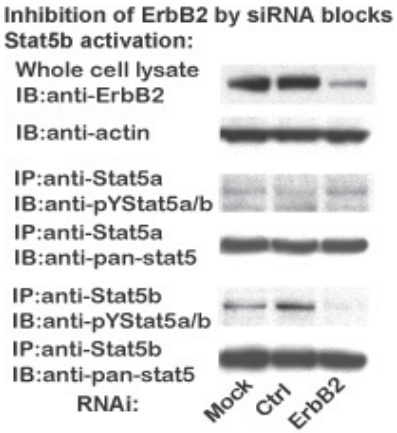


Fig. 2.C.



<u>The dimerization partner of ErbB2 in exponentially growing human prostate cancer cell lines is EGFR.</u> ErbB2 was immunoprecipitated from exponentially growing CWR22Rv (Fig.2.A), LNCaP (Fig.2.B) and DU145 (Fig.2.C) cells and blotted with ErbB2, EGFR, ErbB3 or ErbB4. Whole cell lysates were immunoblotted in parallel to demonstrate protein levels of ErbB2, EGFR, ErbB3 and ErbB4 in each cell line.

Fig.3.



Inhibition of ErbB2 by siRNA blocks Stat5b activation in CWR22Rv prostate cancer cells (Fig.3). The cells were transfected with ErbB2 siRNA and with scrambled siRNAs as control. Immunoblotting of whole cell lysates indicates effective down-regulation of ErbB2 protein expression by ErbB2 siRNA compared to the controls. Stat5a and Stat5b were immunoprecipitated and blotted with phospho-Stat5a/b mAb, filters were stripped and-reblotted with anti-Stat5a/b mAb

The expression of the members of the EGF receptor family in human prostate cancer cells (Fig.4). EGFR is highly expressed in PC3, DU145 and LNCaP cells, while the expression of EGFR is weak in CWR22Rv cells. ErbB2 proteins are expressed in PC3, CWR22Rv, DU145 and LNCaP cells. In addition, ErbB3 is highly expressed in CWR22Rv, DU145 and LNCaP cells. Finally, ErbB4 is only expressed in CWR22Rv cells but not in PC3, DU145, or LNCaP human prostate cancer cells.

Fig.4.

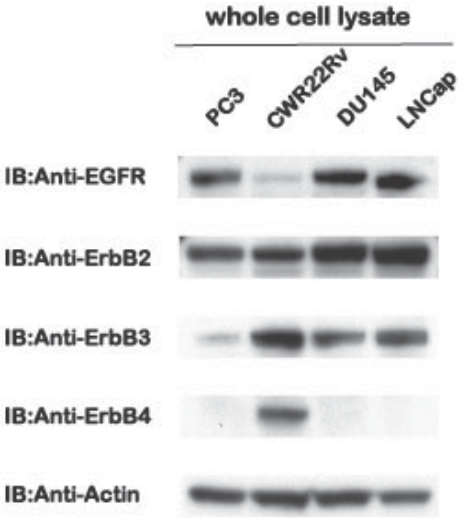
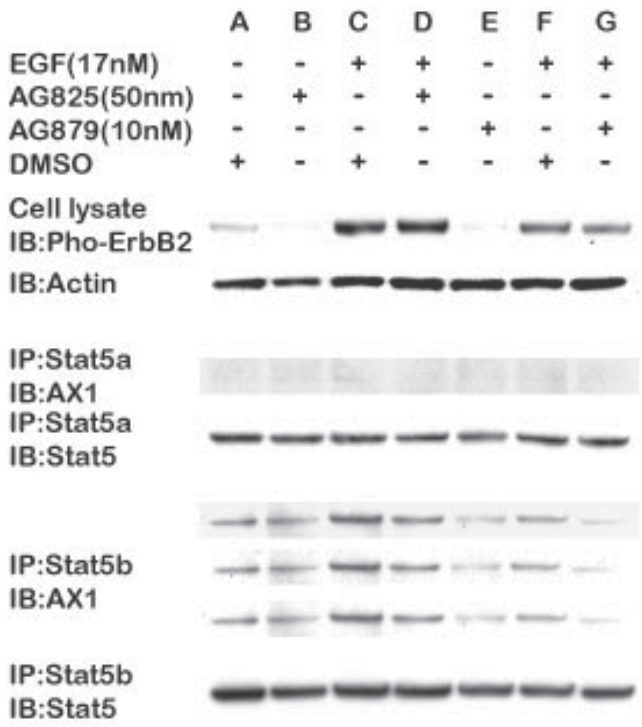
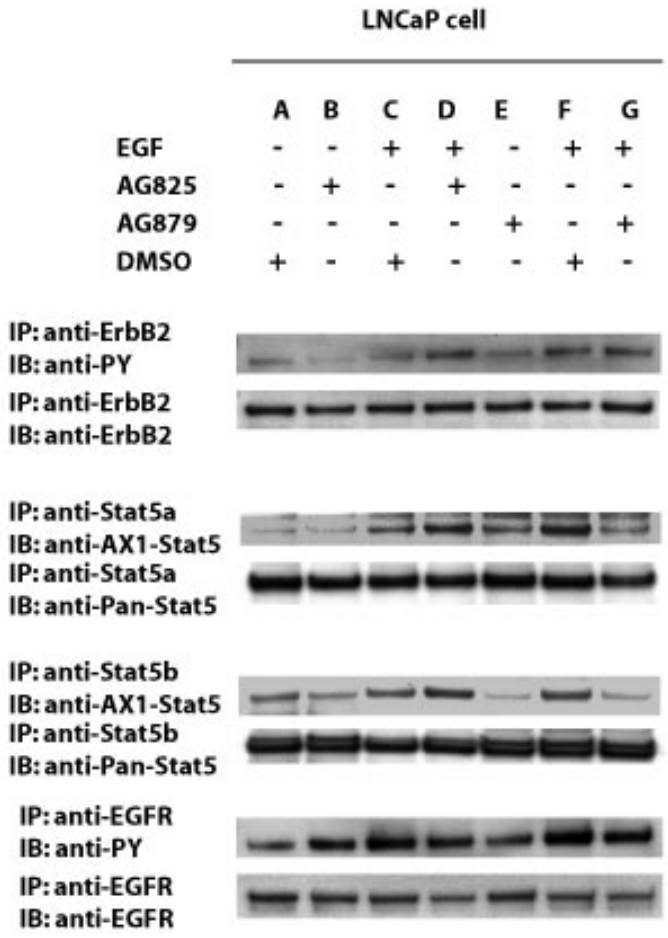


Fig.5.



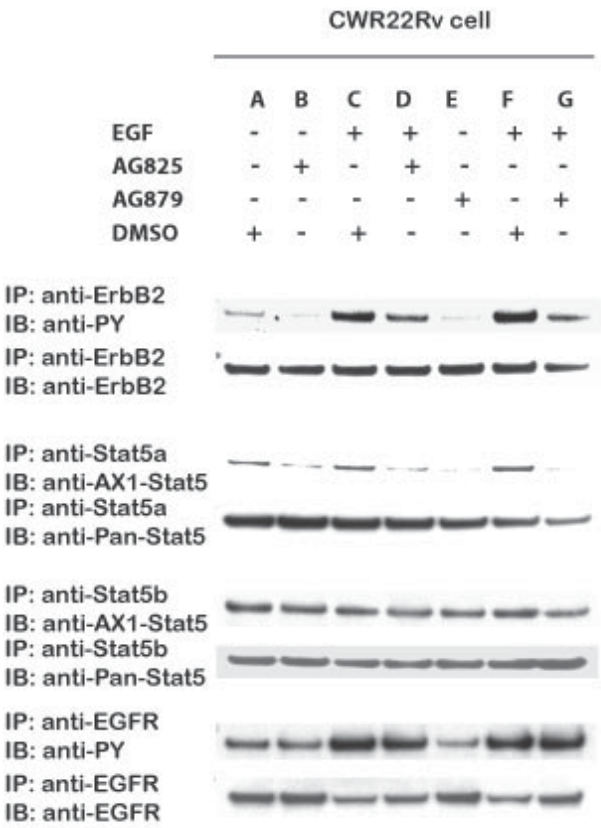
The pharmacological ErbB2 inhibitors block the activation of Stat5a/b in DU145 human prostate cancer cells (Fig. 5). DU145 human prostate cancer cels were exponentially grown and treated with the ErbB2 inhibitors AG825 or AG879 at 50 nM or 10 nM concentrations, respectively, for 48 h. The cells were harvested and Stat5a and Stat5b were immunoprepcipitated and immunoblotted with anti-phospho-Stat5a/b polyclonal antibodies. In addition, ErbB2 levels and phosphor-ErbB2 levels were analyzed from the whole cell lysates by Western blotting.

Fig.6.



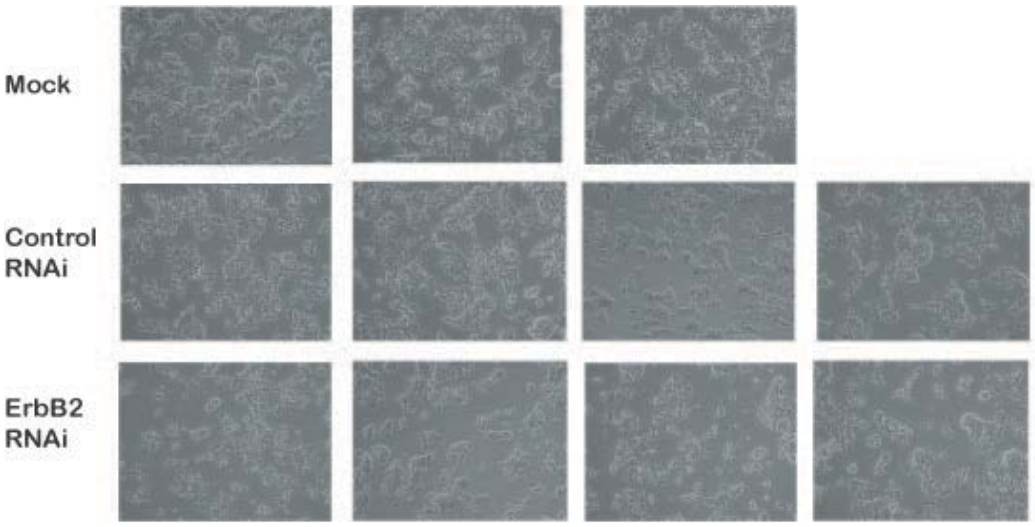
<u>The pharmacological ErbB2 inhibitors block the activation of Stat5a/b in LNCap human prostate cancer cells (Fig. 6).</u> LNCaP human prostate cancer cels were exponentially grown and treated with the ErbB2 inhibitors AG825 or AG879 at 50 nM or 10 nM concentrations, respectively, for 48 h. The cells were harvested and Stat5a and Stat5b were immunoprepcipitated and immunoblotted with anti-phospho-Stat5a/b polyclonal antibodies. In addition, ErbB2 levels and phosphor-ErbB2 levels were analyzed from the whole cell lysates by Western blotting.

Fig.7.

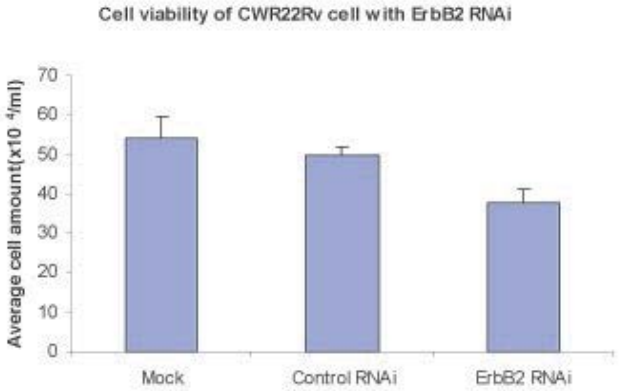


The pharmacological ErbB2 inhibitors block the activation of Stat5a/b in CWR22Rv1 human prostate cancer cells (Fig. 7). CWR22Rv1 human prostate cancer cels were exponentially grown and treated with the ErbB2 inhibitors AG825 or AG879 at 50 nM or 10 nM concentrations, respectively, for 48 h. The cells were harvested and Stat5a and Stat5b were immunoprepcipitated and immunoblotted with anti-phospho-Stat5a/b polyclonal antibodies. In addition, ErbB2 levels and phospho-ErbB2 levels were analyzed from the whole cell lysates by Western blotting.

Fig.8.



Pictures was taken after 72 hours of CWR22Rv cell transfection by control siRNA or ErbB2 RNAi.
Cell counting and picture with the same well of the cell and at the same time.



	Mock	Control RNAi	ErbB2 RNAi
Average cell amount ($x10^4$/ml)	54	50	38

<u>Inhibition of ErbB2 by lentiviral expression of ErbB2 shRNA induces death of CWR22Rv human prostate cancer cells (Fig. 8).</u> CWR22Rv1 human prostate cancer cels were exponentially grown and treated with the ErbB2shRNA lentivirus for 72 h. The cell viability was determined by the MTT assay and cell counting. In conclusion, inhibition of ErbB2 blocks Stat5 activation and decreases viability of human prostate cancer cells.

Next, the investigators generated tissue microarrays of clinical prostate cancers from patients treated by either radical prostatectomy or by watchful waiting. The demographic information of the patients samples was collected (Table1) and the tissue microarrays were immunostained for nuclear Stat5a/b (Fig. 9).

Fig. 9

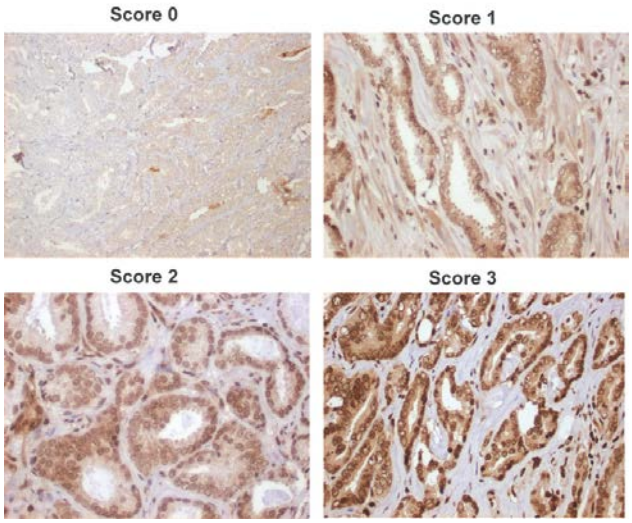


Figure 9. Immunohistochemical detection of Stat5a/b in prostate cancer. Paraffin-embedded sections of prostate cancer tissue microarrays were immunostained with a monoclonal anti-Stat5a/b antibody and biotin-streptavidin amplified peroxidase antiperoxidase immunodetection. 3,3'-Diaminobenzidine was used a s chromogen and Mayer's hematoxylin as counterstain. Individual prostate tissue microarray cores in both Material I and II were scored for nuclear Stat5a/b levels on a scale from 0 to 3, where 0 represented negative (*top*), 1 weak (*second from top*), 2 moderate (*second from bottom*) and 3 (*bottom*) strong immunostaining. The final score for each patient was the maximum of the individual core scores.

Table 1. Characteristics of the prostate cancers in Materials I and II.

	Material I				Material II			
Variables	**Total number of prostate cancers (n=632)**		**Cancers with Stat5a/b expression status (n=562)**		**Total number of prostate cancers (n=419)**		**Cancers with Stat5a/b expression status (n=106)**	
	n	Median (range)	n	Median (range)	n	Median (range)	n	Median (range)
Age at operation, years	632	63 (40-74)	562	63 (40-74)	419	73.6 (51-95)	106	73.9 (51-88.1)
Follow-up time, years	629	7.3 (0.16-24.7)	562	7.3 (0.16-24.7)	419	5.2 (0-25)	106	5.3 (0.1-18.2)
RFS follow-up, years	627	3.7 (0-19.4)	560	3.6 (0.1-19.4)				
Preoperative PSA µg/L	584	8.0 (0.58-290.0)	523	8.2 (0.58-290)				
RFS, follow-up time, years	627	11.4 (8.8,19.4)*	560	9.9 (6.8,-)*				
Tumor size	579	15 (1-100)	521	15 (1-100)				
		n (%)		**n (%)**		**n (%)**		**n (%)**
Gleason score								
4		14 (2.2)		7 (1.3)		14 (3.3)		1 (0.9)
5		49 (7.8)		40 (7.1)		84 (20.1)		28 (26.4)
6		179 (28.3)		159 (28.3)		110 (26.3)		20 (18.9)
7		252 (39.9)		232 (41.3)		72 (17.2)		16 (15.1)
8		83 (13.1)		78 (13.9)		50 (11.9)		13 (12.3)
9		32 (5.1)		31 (5.5)		68 (16.2)		24 (22.6)
10		-		-		21 (5.0)		4 (3.7)
unknown		23 (3.6)		15 (2.7)				
Stage								
pT1		5 (0.8)		5 (0.9)	T1a	73 (17.4)		27 (25.4)
pT2		327 (51.7)		279 (49.6)	T1b	137 (32.7)		28 (26.4)
pT3		276 (43.7)		259 (46.1)	T2	107 (25.5)		17 (16.0)
pT4		1 (0.2)		1 (0.2)	T3	81 (19.3)		29 (27.3)
					T4	13 (3.1)		4 (3.9)
unknown		23 (3.6)		18 (3.2)	unknown	8(1.9)		1 (0.9)
Extra-capsular extension								
Yes		252 (39.9)		237 (42.2)				
No		353 (55.9)		303 (53.9)				
Unknown		27 (4.3)		22 (3.9)				
Positive surgical margins								
Yes		240 (38.0)		216 (38.4)				
No		377 (59.7)		337 (60.0)				
Unknown		15 (2.4)		9 (1.6)				
Seminal vesicle invasion								
Yes		84 (13.3)		81 (14.4)				
No		537 (85.0)		474 (84.3)				
Unknown		11 (1.7)		7 (1.3)				
Lymph node involvement								
Yes		30 (4.8)		29 (5.2)				
No		588 (93.0)		522 (92.9)				
Unknown		14 (2.2)		11 (2.0)				
Stat5a/b nuclear staining score								
0		133 (21.0)		133 (23.7)		11 (2.6)		10 (9.4)
1		182 (28.8)		182 (32.4)		26 (6.2)		24 (22.6)
2		196 (31.0)		196 (34.9)		53 (12.7)		51 (48.2)
3		51 (8.1)		51 (9.1)		26 (6.2)		21 (19.8)
Unevaluable		70 (11.1)		-		303 (72.3)		-

Fig. 10

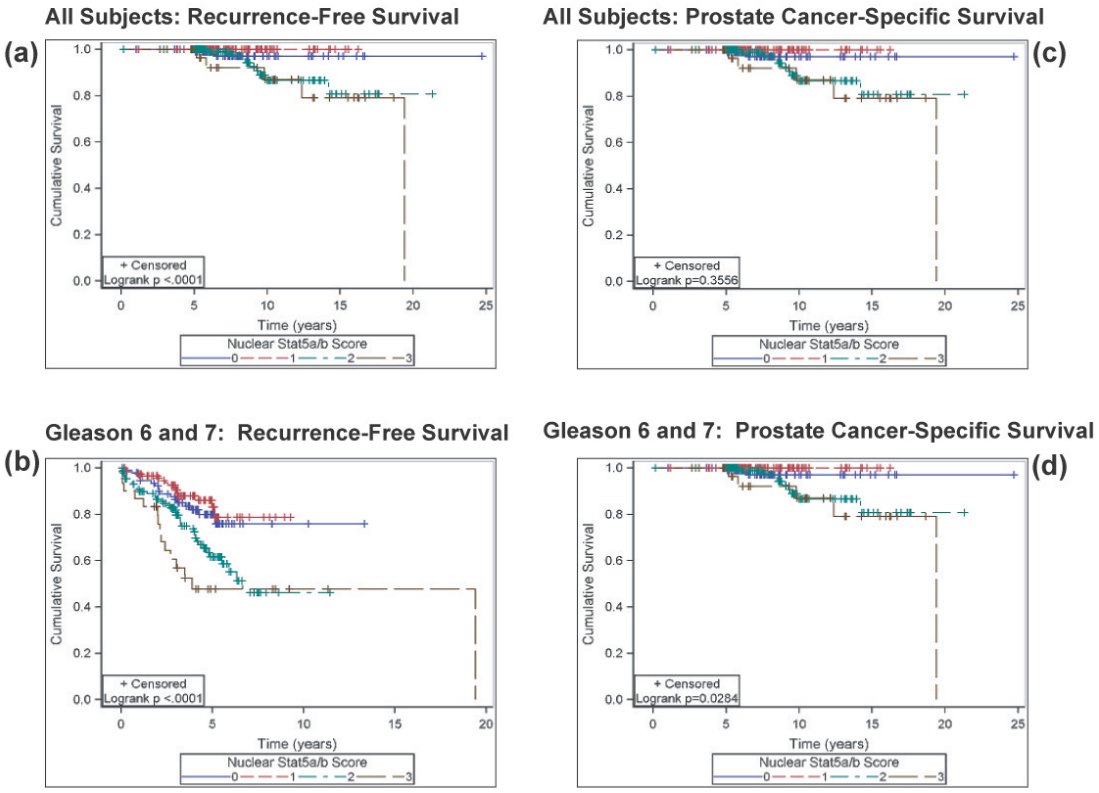


Figure 10. High levels of nuclear Stat5a/b expression predicts early lethal disease recurrence of prostate cancer after radical prostatectomy (Material I). Kaplan-Meier analyses indicating that high nuclear Stat5a/b expression predicts shorter recurrence-free survival in all patients regardless of Gleason (Gl.) scores (n= 560) as well as in intermediate Gl. score prostate cancer patients (n=379) (a and b). In addition, high nuclear Stat5a/b expression in prostate cancer after surgical treatment predicted shorter prostate cancer-specific survival (c and d).

The investigators show that in patients treated by radical prostatectomy, high nuclear Stat5a/b predicted early disease recurrence in both univariate ($p<0.0001$) and multivariate (HR=0.62; $p<0.01$) analysis in the entire cohort, and in intermediate Gleason (Gl.) score (6 and 7) patients by univariate analysis ($p<0.0001$) and multivariate analysis (HR=0.55; $p=0.017$) (Fig.10). In addition, high nuclear Stat5a/b predicted early Prostate cancer-specific death after radical prostatectomy in intermediate Gl. score prostate cancers ($p=0.028$) by univariate analysis (Fig.10).

In the second group of patients treated by watchful waiting, elevated nuclear Stat5a/b expression was associated with early prostate cancer-specific death (p=0.0453) by univariate analysis (60% increased risk of death; p=0.034) (Fig.11). In conclusion, elevated nuclear Stat5a/b was an independent predictor of increased risk of therapy failure in prostate cancers treated by radical prostatectomy including patients with intermediate Gl. Score prostate cancers. High nuclear Stat5a/b expression predicted early prostate cancer-specific death in patients treated by watchful waiting (Fig.11).

Fig. 11

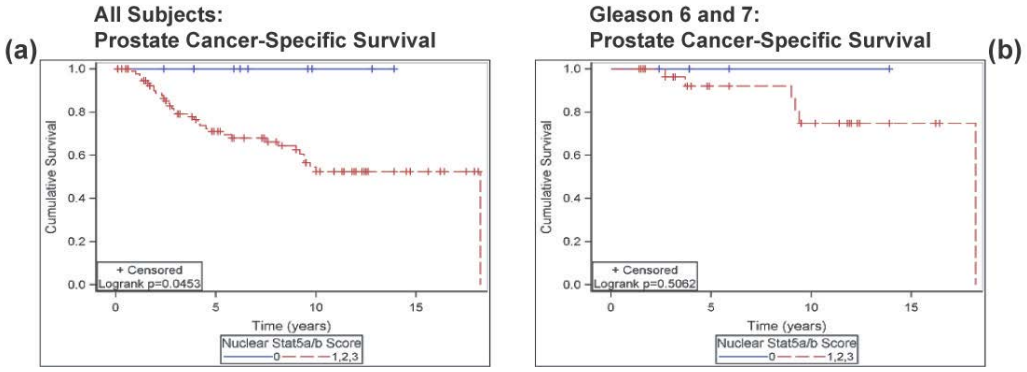


Figure 11. High levels of nuclear Stat5a/b expression in prostate cancer predicts shorter prostate cancer specific-survival in patients treated with watchful waiting (Material II). Kaplan-Meier curves for prostate cancer-specific survival according to negative (top curve) or positive (bottom curve) Stat5a/b activation status in all patients regardless of Gl. score (n=106) (a) and in Gl. score 6 and 7 patients (n=36) (b).

The investigators have tested and set up immunostaining conditions for an anti-ErbB2 antibody (Fig.12) after testing three antibodies from different vendors. The next step is to immunostain the tissue microarrays immunostained for nuclear Stat5 using the antibody recognizing ErbB2, score the staining of individual prostate cancers and carry out the statistical survival analyses.

The investigators have immunostained with anti-ErbB2 antibody the tissue microarrays analyzed previously for predictive significance of Stat5. Importantly, the investigators have conducted extensive survival analyses.

Fig. 12

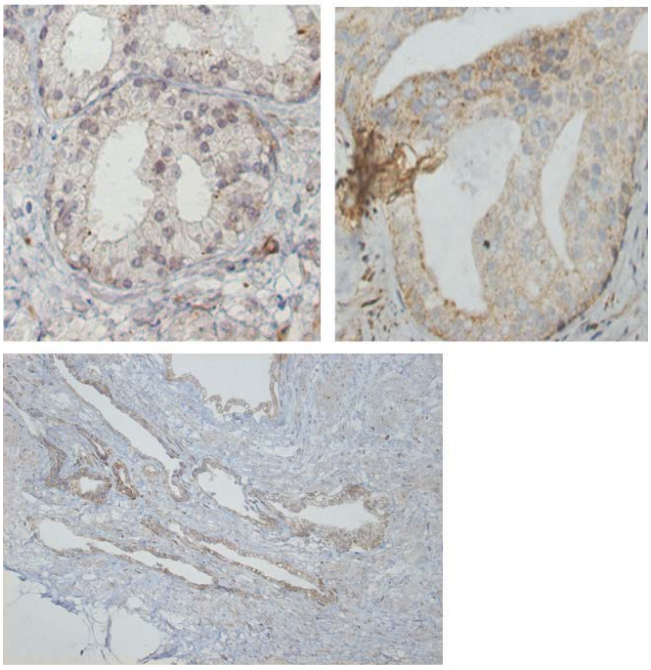


Figure 12. Immunostaining of ErbB2 in clinical prostate cancers. Paraffin-embedded sections of prostate cancer tissue microarrays were immunostained with a monoclonal anti-ErbB2 antibody and biotin-streptavidin amplified peroxidase antiperoxidase immunodetection. 3,3'-Diaminobenzidine was used a s chromogen and Mayer's hematoxylin as counterstain.

Table 2. Characteristics of the prostate cancers in Material I with ErbB1 (HER2) status.

Variable		***Subjects with HER2 Status (N=300)***	
		n	Median (range)
Age at operation, years			63 (40-74)
Follow-up time			7.3 (0.16-24.7)
BRS follow-up time, years			3.7 (0,17.5)
Preoperative PSA μg/L			8.0 (0.58-290.0)
Tumor size			15 (1,100)
		N (%)	
Gleason Score			
	4	2 (0.7)	
	5	22 (7.3)	
	6	93 (31.0)	
	7	127 (42.3)	
	8	36 (12.0)	
	9	12 (4.0)	
	unknown	8 (2.7)	
Gleason Grade			
	2+2	2 (0.7)	
	2+3	3 (1.0)	
	2+4		
	2+5		

	3+1	
	3+2	19 (6.3)
	3+3	92 (30.7)
	3+4	85 (28.3)
	3+5	7 (2.3)
	4+2	2 (0.7)
	4+3	40 (13.3)
	4+4	23 (7.7)
	4+5	11 (3.7)
	5+2	
	5+3	6 (2.0)
	5+4	2 (0.7)
	unknown	8 (2.7)
pT		
	1	-
	2	163 (54.3)
	3	120 (40.0)
	4	1 (0.3)
	unknown	16 (5.3)
Extra-capsular extension		
	Yes	104 (34.7)
	No	176 (58.7)
	Unknown	20 (6.7)
Positive surgical margins		
	Yes	117 (39.0)
	No	178 (59.3)
	Unknown	5 (1.7)
Seminal vesicle invasion		
	Yes	30 (10.0)
	No	265 (88.3)
	Unknown	5 (1.7)
Lymph node involvement		
	Yes	7 (2.3)
	No	285 (95.0)
	Unknown	8 (2.7)
Perineural invasion		
	Yes	187 (62.3)
	No	91 (30.3)
	Unknown	22 (7.3)
Stat5 nuclear score		
	2,3	110 (36.7)
	0,1	161 (53.7)
	Unevaluable	29 (9.7)
Adjuvant hormone therapy		
	Yes	14 (4.7)
	No	286 (95.3)
Adjuvant radiation therapy		
	Yes	50 (16.7)
	No	250 (83.3)
Salvage hormone therapy		
	Yes	17 (5.7)
	No	283 (94.3)
Salvage radiation therapy		
	Yes	20 (6.7)
	No	280 (93.3)

Figure 13. High levels of ErbB2 expression predicted early lethal disease recurrence of prostate cancer after radical prostatectomy (Material I). Kaplan-Meier analyses indicating that ErbB2 expression predicts shorter recurrence-free survival in prostate cancer.

Figure 13. Biochemical recurrence-free survival by HER2 Status

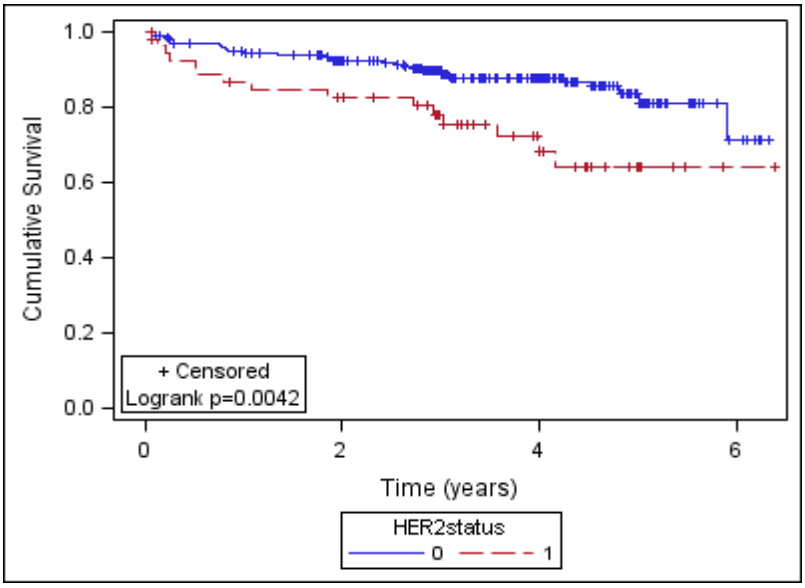


Figure 14. High levels of nuclear Stat5 expression in the cohort with both Stat5 and ErbB2 status, high nuclear Stat5 predicted early lethal disease recurrence of prostate cancer after radical prostatectomy (Material I). Kaplan-Meier analyses indicating that nuclear Stat5 expression predicts shorter recurrence-free survival in prostate cancer.

Figure 14. Biochemical recurrence-free survival by Stat5 score.

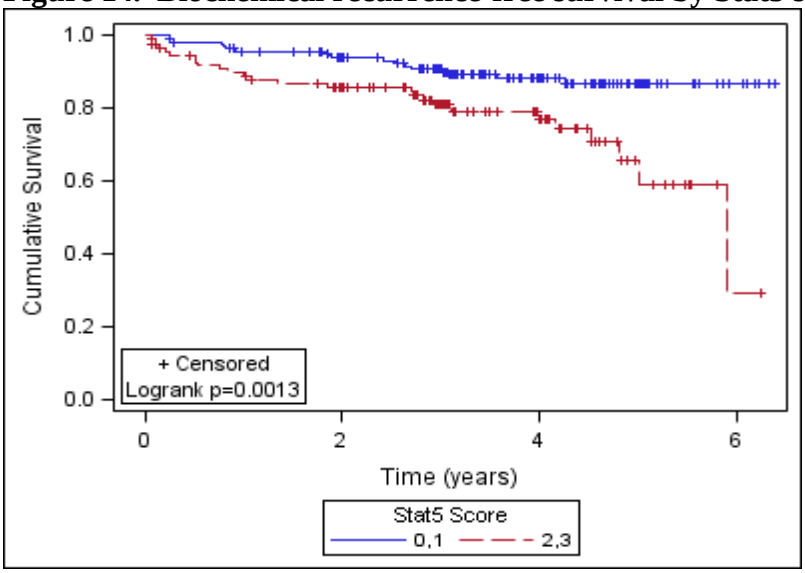
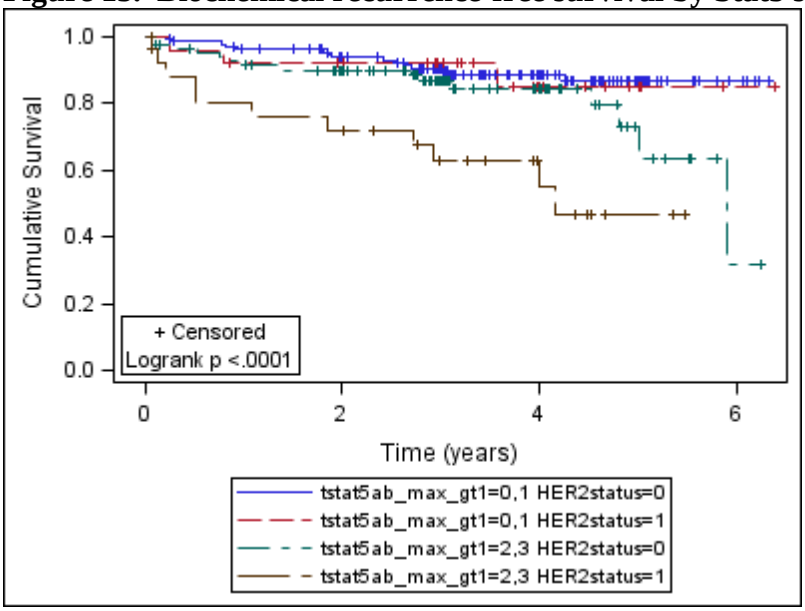


Figure 15. High levels of both nuclear Stat5 and ErbB2 expression (brown line) predicted early lethal disease recurrence of prostate cancer after radical prostatectomy (Material I) more effectively than nuclear Stat5 or high ErbB2 alone. Kaplan-Meier analyses indicating that combined expression of nuclear Stat5 and ErbB2 predict shorter recurrence-free survival in prostate cancer better than nuclear Stat5 expression alone or ErbB2 expression alone.

Figure 15. Biochemical recurrence-free survival by Stat5 score and HER2 status.



18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__x___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
____x_No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
____X_No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

ErbB2-Stat5 Signaling Axis Promotes Prostate Cancer Cell Viability and Clinical Progression Dagvadorj A, Talati P, Leiby B, Mirtti T, Shen F, Nevalainen MT. To be submitted, 2013.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The results of the conducted studies show that high levels of active Stat5 in prostate cancer predict both early disease recurrence and prostate cancer-specific death. Importantly, combined expression of active Stat5 and ErbB2 provided even better predictive information on early disease recurrence than active Stat5 alone or ErbB2 expression alone in prostate cancer.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The results of the conducted studies show that high levels of active Stat5 in prostate cancer predict both early disease recurrence and prostate cancer-specific death. Importantly,

combined expression of active Stat5 and ErbB2 provided even better predictive information on early disease recurrence than active Stat5 alone or ErbB2 expression alone in prostate cancer.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X___

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes__ No__

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes__ No__
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No__

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes No__

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X___

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
NEVALAINEN, **Marja T., MD, PhD**	Associate Professor of Cancer Biology, Medical

eRA COMMONS ID MNEVALAINEN	Oncology and Urology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Turku, Turku, Finland	B.M.	1988	Medicine
University of Turku, Turku, Finland	M.D.	1992	Medicine
Health Central of Loimaa, Loimaa, Finland	Licensed Physician	1993	Clinical Residency, Medicine
University of Turku, Turku, Finland	Ph.D.	1997	Cell Biology

A. PERSONAL STATEMENT

Dr. Nevalainen's expertise area is in Jak-Stat signaling in prostate cancer. Her early work identified Stat5 as the key signaling protein that mediates the effects of prolactin in prostate cancer. her group showed, for the first time, that prolactin is locally produced growth factor in human prostate tissue, and that both prolactin and active Stat5 expression is associated with high histological grades of clinical prostate cancers. Dr. Nevalainen's research group has provided the proof-of-principle that Stat5a/b is a therapeutic target molecule in prostate cancer and further validated the concept in numerous prostate cancer model systems. Her group showed that Stat5 effects are independent of the androgen receptor and Stat3, and that Stat5 promotes prostate cancer metastases *in vivo*. They showed that elevated nuclear Stat5 protein levels predict early prostate cancer recurrence and early prostate cancer-specific death. Their recent work indicates that Stat5 gene locus undergoes somatic amplification during prostate cancer progression to castrate-resistant disease. Moreover, their data show that Stat5 inhibition potently inhibits castrate-resistant growth of prostate cancer *in vivo*. Over the years, Dr. Nevalainen's group has developed several *in vivo* model systems for castrate-resistant and metastatic prostate cancer (CWR22Pc), and an *ex vivo* explant organ culture system for drug-responsiveness testing of human prostate cancer.

B. POSITIONS AND HONORS

Positions and Employment:

1993	Clinical Residency, Primary Care Unit of Loimaa, Loimaa, Finland.
1994-1997	Instructor, Dept. Cell Biology, University of Turku, Turku, Finland.
1997-1998	Finnish Academy of Science: Visiting Fellow, Uniformed Services University of Health Sciences.
1998-1999	WHO-IARC-Fellow (World-Health Organization's International Agency for Research on Cancer), United States Military Cancer Institute, USUHS, Bethesda, MD.
2000-2002	Visiting Fellow, United States Military Cancer Institute, USUHS, Bethesda, MD.
2002-2003	Assistant Professor, Dept. Cell Biology and Lombardi Cancer Center, Georgetown University, Washington, DC.
2003-2006	Assistant Professor, Dept. of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC.
2006-present	Associate Professor of Cancer Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania.
2006-present	Associate Professor of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania.
2007-present	Associate Director of Education, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania.
2007-present	Vice Chairman – Education, Dept. of Cancer Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania.
2007-present	Member of the Executive Committee of Kimmel Cancer Center, Thomas Jefferson University

2009-present	Member of the Research Advisory Committee of Thomas Jefferson University
2009-2010	Member of the Professional Faculty Advisory Committee of Thomas Jefferson University
2009-present	Associate Professor of Urology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania.
2009-present	Co-Director: Greater Philadelphia Prostate Cancer Working Group