



## Deliverable No. D3.2

# Report on scenarios and data from other cancer types for usage by the CHIC infrastructure

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PP	Restricted to other programme participants (including the Commission Services)	
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**ABSTRACT:**

This deliverable describes the scenarios and data from cancer types different from those on which the CHIC project is mainly focused (Nephroblastoma NB, Glioblastoma GB and Lung NSCLC), which the goal of anticipating and foreshadowing the infrastructure requirements and the expected issues related to translation and usage in clinical care.

The choice of the cancer type (i.e. Prostate Cancer) has been driven by a number of nice features: it is a very diffuse cancer which has a slow progression, it can be monitored by dosing a specific biomarker using a simple and cheap procedure alternative to imaging, it is currently cured by using different therapeutic approaches (surgical, radiological and chemo-hormonal).

All the previous features make Prostate Cancer (PCa) and ideal candidate for testing the CHIC infrastructure needed for promoting clinical networking. Moreover, we can properly manage the ethical and privacy – related issues. We can collect and cure (massive) clinical data flow and sharing them within the scientific community. We can organize efficient database structures allowing statistical and mathematical validation. We can design hypo-models predicting the natural history as well as the response to a variety of therapeutic approaches. Finally, we can envisage exploitation and dissemination future activities.

<sup>1</sup> R=Report, P=Prototype, D=Demonstrator, O=Other

<sup>2</sup> PU=Public, PP=Restricted to other programme participants (including the Commission Services), RE=Restricted to a group specified by the consortium (including the Commission Services), CO=Confidential, only for members of the consortium (including the Commission Services)

**KEYWORD LIST:**

Prostate Cancer (PCa), clinical networking, ethical and privacy – related issues, clinical data collection, database structures, statistical and mathematical validation, hypo-models, natural history, therapy, exploitation and dissemination

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## 1 Executive Summary

In the mainframe of Prostate Cancer (PCa), which is a very diffused pathology addressed by many different therapeutic approaches, we tried to define a scenario in which the main features of the CHIC infrastructure may be foreseen.

After a short presentation of the clinical network in Regione Piemonte that has been built and supported within the CHIC project, attention will be focused on the following points:

1. the collection and storage of clinical data (EUREKA1 and EUREKA2 studies),
2. the appropriate ethical and technological tools for effective clinical repositories
3. the appropriate ICT and computational tools for an efficient model evaluation/validation oriented database
4. the proposal of predictive hypomodels and their clinical evaluation/validation
5. the feasibility of clinically oriented hypermodels for PCa
6. exploitation and dissemination issues

A large part of the results reported in the present deliverable has been published and/or communicated in international Conferences and Papers, as shown in Fig. 1.1

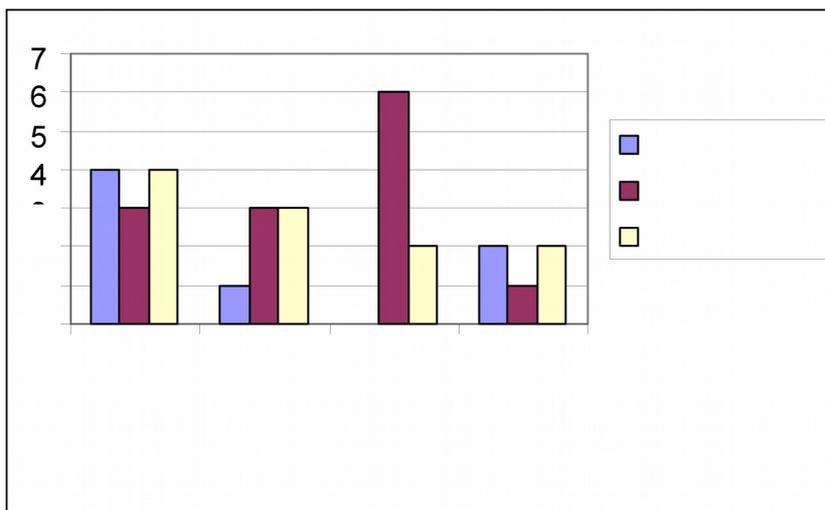


Fig. 1.1 Communications during the previous months.

## 2 Introduction

### 2.1 Purpose of this document

This document defines and describes the scenario and data for other cancer types, specifically for Prostate Cancer, for usage by the CHIC infrastructure.

The concept of scenario within CHIC is a very general one, as shown by the following picture:

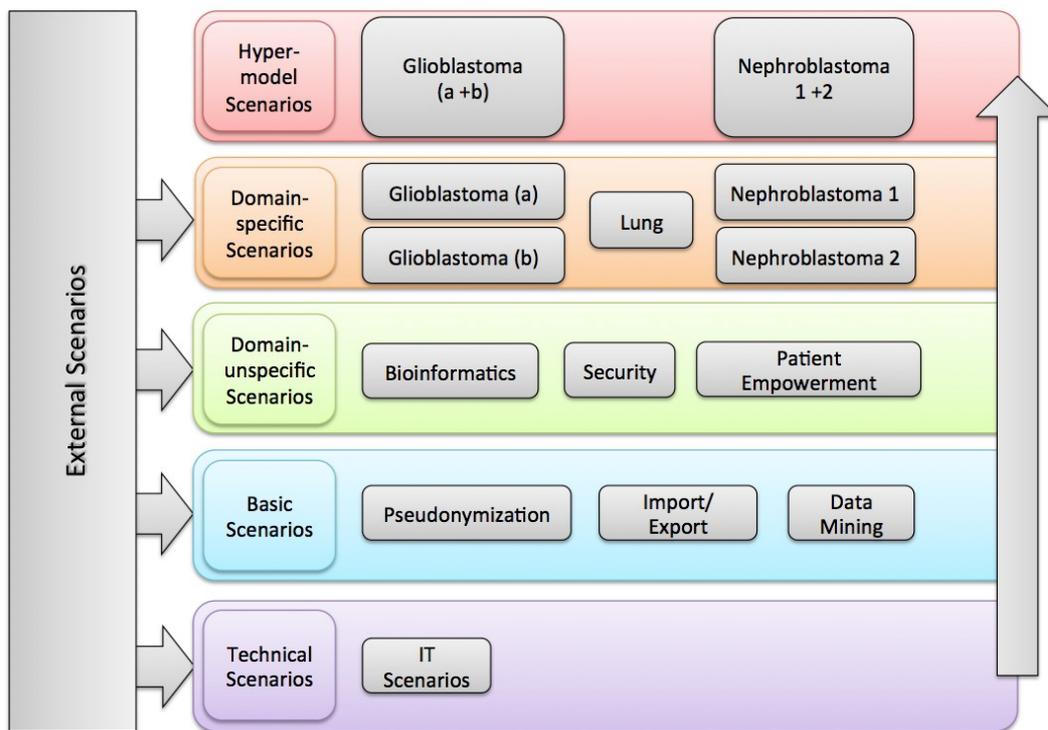


Fig. 2.1: Levels of scenarios giving examples on all levels. Interoperability and standards will allow the integration of external scenarios

Each scenario needs to be translated into a use case via iteration between clinicians and all other stakeholders involved in the project.

In the case of Prostate Cancer, the Basic Scenario, the Domain-unspecific Scenario, the Domain-specific Scenario and the hyper-model Scenario have been involved within the general approach of a **clinically driven Scenario**.

In particular, the overall clinically driven scenario, the Basic and the Domain-unspecific Scenarios will be investigated in Chapter 3, while the Domain-specific and the hyper-model ones are detailed in Chapter 4 and 5.

### 3 Prostate cancer as ideal candidate for testing CHIC infrastructure

#### 3.1 Introduction

Prostate cancer (PCa) is a slow-proliferating adenocarcinoma with a steadily increasing incidence related to ageing. It is the most common cancer in men and the second most common cause of death from tumors in the male population. In 3 cases on 4 it can be successfully cured, otherwise relapse occurs both locally or inducing distant metastasis.

Like breast cancer for women, hormonal drive is crucial for proliferation and for survival, making PCa highly integrated within the endocrine homeostasis of the whole body.

Together with diffusion and slow proliferation, some other issues make PCa an ideal candidate for modelling and for testing CHIC infrastructures:

1) an easily assessable and cost effective blood marker, the PSA (Prostate Specific Antigen), secreted in the human body exclusively by the prostatic tissue, is currently evaluated in men when PCa is suspected. It is useful for diagnostic purposes and highly reliable for relapse evaluations. Although also gene mutation, protein expression patterns and miRNA regulation are under study and will further disclose prostate cancer physiopathology, prognostic classification and treatment response evaluation, only for PCa, a biomarker is already available on large scales. For prostatic patients the collection of PSA values may afford a reliable monitoring of tumor growth with no need of specific and repeated follow-up through imaging;

2) the feasibility of needle biopsy and the well consolidated Gleason grading system proposed almost 50 years ago makes it possible a histological analysis of PCa cells i.e. *bioptic Gleason Score (bGS)* which, following radical prostatectomy on the whole prostate specimen, will be corroborated by the *pathologic Gleason Score (pGS)*. (2005 ISUP Modified Gleason System) [1]. Both the bGS and the availability of several PSA evaluation add information to the traditional TNM staging system assigned at diagnosis through clinical and radiological exams, i.e. *clinical staging*. For Prostate cancer staging is performed according to American Joint Committee on Cancer (AJCC) 2010, Seventh Edition.

3) Prostate cancer is managed by many clinicians in an interdisciplinary way. Practically all the oncology specialists and hospital services have to deal with prostate cancer: Radiotherapists, Urologists and Medical Oncologists for treatment, Pathologists and Radiologists for diagnosis and staging, Epidemiologists and even General Practitioners for the widespread diffusion in the population and for the co-operation during follow-up. Different therapies are also performed, ranging from Surgery, Radiotherapy and drugs (mainly Androgen Deprivation Therapy, ADT). Guidelines for the clinical use of the various treatment modalities have been proposed by several clinical associations, such as European Association of Urology (EAU) [2,3] for urologists, NCCN (National Comprehensive Cancer Network) [4,5] for radiotherapists and Piedmont Guidelines [6] for the multidisciplinary approach including medical oncologists. In general, surgery (Radical Prostatectomy, RP) is indicated in low and intermediate-risk patients, brachytherapy in low-risk

patients, EBRT (External Beam Radiation Therapy) in all risk patients, joined to ADT in intermediate and high-risk patients; ADT (eventually with palliative RT) is fostered for metastatic disease, while in castration-resistant patients chemotherapy or new drugs are indicated.

These different therapeutic approaches offer a wider range of possibilities to modelers in order to test several hypo- and hyper-models.

### 3.2 Opportunities of clinical networking in Piedmont Region

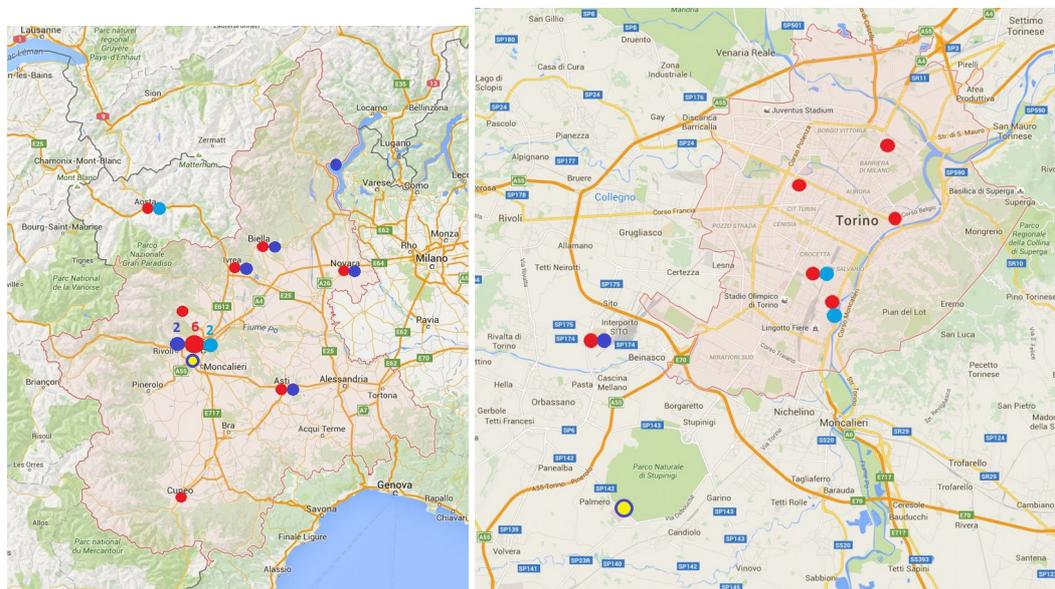
The Piedmont Cancer Registry (RTP) [7] is one of the oldest Cancer Registry in Italy and since 1985 monitors incidence, mortality, prevalence and survival of cancer cases prostate in Turin. A steady increase occurred of more than three times over the last 20 years. The standardized rate (x 100,000 / year) rose from 29.3 in 1985 to 101.3 in 2005, with an average number of 723 new cases diagnosed per year in the 2003-2005.

The risk of having prostate cancer increases significantly with age: the rate (x 100,000) went from about 5, in the age group 45-49, to 800, in the class 75-79 (RTP data 2002-2004 [7]).

Regione Piemonte is equipped to treat the average 8000 new patients per year with a large clinical network. Among the Hospital Divisions we selected those with the largest casuistry.

Following direct invitation, most of them agreed to collaborate to a large clinical data collection aimed at evaluating/validating the model activities promoted within the CHIC research project.

Most of the Centers are in Piemonte- Val d'Aosta (see Fig1) and specifically within the Torino Area (see Fig 2), red dots denote the Urology Dept and blue dots the Radiotherapy Dept.



EUREKA-1 (Radical Prostatectomy)		EUREKA-2 (Radical Radiotherapy)	
Participating Hospital	Town and Unit Director	Participating Hospital	Town and RT Director
Ospedale	Torino	IRCCS	Candiolo

Molinette University	Gontero Prof.		Gabriele MD
San Luigi Gonzaga University	Orbassano Porpiglia Prof.	Ospedale Maggiore University	Novara Krengli Prof.
Ospedale Maggiore University	Novara Terrone Prof.	San Luigi Gonzaga University	Orbassano Ruo Redda Prof.
Ospedale San Giovanni Bosco	Torino Muto MD	Ospedale Civile	Ivrea Girelli MD
Ospedale Santa Croce	Cuneo Arena MD	Ospedale degli Infermi	Biella Moro MD
Ospedale Regionale	Aosta Benvenuti MD	Ospedale Cardinal Massaia	Asti Tessa MD
Ospedale Gradenigo	Torino Randone MD	Istituto Europeo di Oncologia	Milano (outside Piemonte) Orecchia Prof.
Ospedale Cardinal Massaia	Asti Bardari MD	Osp. Santa Chiara University	Pisa (outside Piemonte) Panichi MD
Ospedale Civile / ASL Torino 4	Ivrea / Cirié Annoscia MD		
Ospedale Santissima Trinità	Borgomanero Monesi MD		
Ospedale Maria Vittoria	Torino Moroni MD		
Ospedale Mauriziano	Torino Comi MD		

They all agreed to join two observational multi-centric historic cohort studies: EUREKA-1 regarding patients treated by Radical Prostatectomy (RP) and EUREKA-2 concerning patients treated by External Beam Radiation Therapy (EBRT).

### **3.3 Ethical and privacy-related issues in national and European contest**

The procedures were parallel for the two cohort studies.

The two groups met in Torino in spring 2013 and agreed about:

1. a common protocol for inclusion and exclusion criteria of patients to the studies (see Appendix 1 and 2), which are enrolled consecutively by the structures which join the studies;
2. the definition of a 'leader clinical structure' responsible for the achievement of a common authorisation framework which includes all the participants.

The FPO-IRCCS Cancer Center of Candiolo was selected as leader structure for both studies and Dr. Pietro Gabriele, Director of the Radiation Oncology Division, FPO-IRCCS Cancer Center of Candiolo, was proposed as Principal Investigator.

Consequently, EUREKA-1 and EUREKA-2 studies, were firstly approved by FPO-IRCCS Ethical Committee in July 8<sup>th</sup>, 2013 (see Appendix 3 and 4) and amended as versions 2.0 in November 12<sup>th</sup>, 2014 (see Appendix 5).

Being retrospective, the studies EUREKA, versions 1.2, did not foresee the collection of patients' informed consent according to the Italian laws "Codice in materia di protezione dei dati personali, Allegato A4 - Codice di deontologia e di buona condotta per i trattamenti di dati personali per scopi statistici e scientifici" (provvedimento del Garante n. 2 del 16 giugno 2004); "Autorizzazione al trattamento dei dati idonei a rivelare lo stato di salute e la vita sessuale" (provvedimento del Garante n. 2 del 13 dicembre 2012); and "Autorizzazione generale al trattamento dei dati personali effettuato per scopi di ricerca scientifica" (provvedimento del Garante della Privacy n. 9 del 13 dicembre 2012).

On the contrary, the historic cohort EUREKA studies, versions 2.0, foresee the delivery to patients of the informed consent and of the letter to the General Practitioner, to be signed during future periodic follow-up clinical visits upon the participating Urology and Radiotherapy Divisions.

Besides, the participating centers were allowed to contact Cancer and Civil Registries to update information, e.g. for dead patients.

According to privacy procedures currently used in Italy, pseudo-anonymization is performed by appointing an ID code for each single patient by the local researchers participating to the research. The two lists linking patients' personal data and clinical data belong to the Hospital and are not accessible by the EUREKAs researchers, only clinical data, and not personal data, are sent to the common databases of the coordinating centers. In the common EUREKA databases patients will be anonymous, identified by their ID code only and by no way amenable to their identity.

The anonymized databases will be shared with the coordinating centers (Division of Radiation Oncology, FPO-IRCCS Cancer Center of Candiolo and Medical Physics Laboratory, Neuroscience Department, University of Turin). Data were stocked in anonymous electronic databases, with limited access (checked with username and keyword) exclusively to researchers directly involved in data checking and data analysis.

A dedicated server without any web connections located at Medical Physics Lab, Neuroscience Dept in Torino will hold the input clinical database and all their derivatives in order to use them for modelling evaluation/validation as long as they are required for the CHIC project to be fully completed.

Dr Domenico Gabriele was requested to develop a standard database pattern for both EUREKA1 and EUREKA2 studies to be filled in by the clinical divisions involved in the studies, accounting for the data which are normally quoted in the hospital records and may be available at the

pathology services. Database details include: socio-demographic data, biopsy and staging, therapies, pathology, oncologic outcomes, serologic and clinical follow-up and collateral effects.

Appendix 6 shows the database input variables for EUREKA1 and Appendix 7 for EUREKA2 studies.

### ***3.4 Clinical data collection: from the Hospital repositories to an efficient database for model validation***

#### **3.4.1 Availability of clinical data**

Data collected from hospital records were stocked in two electronic databases (one for EUREKA-1 and one for EUREKA-2) according to different domains required: e.g. proper data format (integer number, number with decimal figures, percentage, text and date in dd/mm/yyyy format) in the dedicated server.

The files are provisionally stored with Microsoft Excel and Microsoft Access data management software; besides, additional copies are recorded for specific applications (STATA, SPSS and SAS statistical software, MATLAB for modelling validation).

#### **3.4.2 Data quality**

The total number of patients included in the database by the participating centers were 4445 and 4275 for EUREKA-1 and EUREKA-2 studies, respectively. Some of these were excluded because they were incomplete according to EUREKA protocols: short follow-up = 907 patients (20% loss) and few pre-treatment parameters = 409 patients (12% loss), respectively. Consequently, the final database included 3538 and 3776 cases for EUREKA-1 and EUREKA-2 studies, respectively. Several issues arose during and after the data collection procedures:

- a) case histories/reports were not always available in electronic format (the switch between paper and electronic data recording in the Italian national health system started extensively only by the early 2000s with great discrepancy between hospitals);
- b) being the studies retrospective, data are incomplete in a significant percentage of patients, mainly in the pathology and follow-up fields (e.g. post-treatment PSA list and cause of death);
- c) data relying on semi-quantitative operator-dependent evaluations are sometimes inhomogeneous (e.g. Gleason Score analysis and the fields related to margin closeness, usually expressed in a qualitative manner rather than in millimeters);
- d) a relevant percentage of scintigraphy exams are not assessable for metastasis evaluation because of doubtful findings, with metastatic status defined after further analysis (usually a focused RX) or sometimes not assessable;
- e) rare copy mistakes (for example in the PSA values' list) or omissions (a few Dose-Volume-Histograms lacking in the case histories).

The applied solutions to overcome these pitfalls were:

- 1) direct access to reports in the pathology units, which are more reliable than the incomplete copies recorded in the clinical urology and radiotherapy units;
- 2) phone calls, fax and e-mails to update follow-up (if patients were dead information was usually asked for to close relatives such as wife / sons or the General Practitioner of patient itself, or retrieved through Cancer and Civil Registries);
- 3) ad-hoc hospital visits if some follow-up were not available for any reason;
- 4) scientific meetings for pathologists and physicians to discuss on common language chosen for the database;
- 5) quality assessment protocol at the end of data collection, to check approximately 10% of the case histories and to homogenize the whole dataset.

Data with missing overcoming 30% were excluded in modeling analysis; besides, at least three PSA values were mandatory to compute PSA Doubling-Time.

An automatic data quality control was also performed after the manual data checking described above. In particular, data were stored in a dedicated server (operative system: Windows 2008 Server) and triggers were programmed on PostgreSQL 9.3, assuring:

- Consistency: format, admitted values and admitted ranges were checked in all the major columns, using triggers during the import and data quality queries after it.
- Completeness: patients without basic information were excluded from the database.

- Usability: derived tables and views were constructed to easily join and compare data. Primary keys were always used. The database is completely anonymous and an export in other file format (e.g. CSV) is possible.

## 4 Predicting (hypo-)models for PCa recurrence after surgery

### 4.1 Introduction

Prostate cancer (PCa) is well known to be one of the most slowly growing tumor, which often affects elderly people who will likely die for other causes. In addition, PCa can be diagnosed and tumor evolution can currently be monitored by dosing the Prostate Specific Antigen (PSA) in serum, which is a safe, non invasive and cheap procedure.

In spite of the former advantages, however, PCa is known to produce local and/or distal recurrences in around a quarter of patients who underwent radical prostatectomy (RP) (see [8]) or radical radiotherapy (RR). Salvage therapies may become very critical for them. In recurrent PCa, after a variable time following the primary therapy (RP or RR), a so-called “biochemical recurrence” (BCR) is observed, with a progressive rise of the PSA values above or at 0.20 ng/ml after RP (see [9]).

An adjuvant therapy is sometimes prescribed just after RP or RR, consisting in various forms of Androgen Deprivation Therapy, which dramatically reduces PSA values but normally fails in controlling the tumor, which usually becomes resistant to therapy.

Predicting the probability of recurrence of PCa after RP is one of the main goals of studies and researches in this field. Roughly speaking, there are two main ways of thinking: one relates the recurrence probability and its timing to the pre-operative tumor characteristics (e.g. Gleason Score, tumor stage, surgical methods [see Figure 4.2 for details] and so on – static models) while the second one investigates the postoperative tumor dynamics mainly based on the PSA growth timing (dynamic models).

Static models are normally validated on huge clinical database, and aim at producing simple and reliable tools for addressing therapeutic decisions. Very popular nomograms have been proposed, starting from the first model of [10], the GPSM (Gleason, PSA, Seminal Vesicle and Margin Status) proposed by [11], the nomogram of Briganti [12] and all their updated versions.

Dynamic models, already proposed by D'Amico [13] and independently by Yorke [14], and further developed by Dimonte[15] focus on the estimation of PSA velocity and doubling time, evaluating the timing of tumor proliferation from serial PSA measurements.

As an alternative to direct evaluation of PSA data dynamics, the use of the Universal Growth Law (UGL) proposed by West et al.[16] and formerly applied to tumors by Guiot et al.[17], may offer a frame for describing PSA (and tumor) growth predicting its clinical outcome. In particular, two parameters of the equation should be analysed: the growth parameter alpha and the carrying capacity (P in the following equations). According to [16,17], the carrying capacity should be set depending on the physical problem, i.e. the maximum value achievable by the population taken into account.

Based on the PCa tumor volumes measured along the natural history of PCa [14,17], the growth parameter alpha estimated by the model is about  $0.42 \text{ g}^{0.25} / \text{day}$ . If a few surviving PCa cells start proliferating and producing a detectable quantity of PSA after RP, we assume that the PSA detected in the patient serum should follow the same 'universal law' with a value of alpha which reflects its actual proliferation. Accordingly to West and colleagues, alpha is the ratio between the metabolic energy and the energy needed for duplication of a given cell type. In case of PSA production, it is expected to increase when cells actively produce PSA and reproduce themselves. Moreover, it should decrease when cells produce small amounts of PSA and duplicate slowly.

The parameter alpha can then be added to the standard clinical parameters into a statistical multi-parametric model to estimate timing of recurrence on retrospective data. Moreover, in order to devise a robust and easily manageable tool for clinicians, we investigated how many PSA values are needed to make a good prediction. Predictability of the growth parameter based on the first 3 detectable PSA measurements are investigated to study the feasibility of a rapid estimation procedure.

## 4.2 Clinical data

Starting from EUREKA1 study, we considered the patients with at least 4 PSA values reported in their follow up. The 27 % of them had a relapse. Most of them did not undergo any adjuvant therapy. Among those undergoing adjuvant therapies, patients had ADT, adjuvant RT or both.

We excluded the patients whose first PSA after surgery was larger than 0.2 ng/ml since their primary tumor was not properly removed or metastasis were already present.

Finally, we considered only the patients treated with the radical prostatectomy, excluding the nerve-sparing surgeries because of possible prostate cells remnants.

Our sample is composed by 212 patients without adjuvant therapies and 40 patients with only adjuvant ADT for at least six months.

## 4.3 Math procedure

According to the model presented by Thompson et al. [9], we suppose that the PSA dosage  $p$  collected at time  $t$  reflect the actual tumor mass at that stage and tumor growth can be described as follow:

$$\frac{dp}{dt} = \alpha p^{\frac{3}{4}} \left[ 1 - \left( \frac{p}{P} \right)^{\frac{1}{4}} \right]$$

where  $p$  is the PSA value (in ng/ml),  $P$  is its maximum value reached by the population. In our case  $P = 100$  ng/ml,  $t$  is the timing of the measurement expressed in months after surgery and alpha is the growth parameter for PSA.

Physical data can be re-normalized following simple calculations (see [9] for the details), in terms of rescaled tumor fraction  $r = (p/P)^{1/4}$  and rescaled time

$$\tau = \frac{\alpha t}{4P^{\frac{1}{4}}} - \ln \left( 1 - \left( \frac{p_0}{P} \right)^{\frac{1}{4}} \right)$$

where  $p_0$  is the initial value of the series. Far from being mathematical tricks, the rescaled units allows us a quick comparison between our experimental data and the parameterless universal curve

$$r = 1 - e^{-\tau}$$

obtained by substituting  $r$  and alpha in the previous equations.

Secondly, we can evaluate the alpha parameter value deterministically by averaging the single alpha values corresponding to the measured PSA:

$$\alpha = \frac{1}{n} \sum \alpha_i = \frac{1}{n} \sum 4P^{\frac{1}{4}} \frac{\log(1 - \frac{p_0}{P}) - \log(1 - \frac{p_i}{P})}{t_i}$$

where  $p_i$ ,  $t_i$  are the non null  $i$ th PSA values and the times of the measurement respectively and  $n$  is the size of the sample. Note that in some cases this method failed and alpha results equal to zero, hence the time to relapse can not be estimated.

In case of adjuvant ADT, which tends to depress the PSA value and the tumor volume during its action, we calculated alpha as the average of  $\alpha_i$  from the set of PSA values taken after the end of it.

### 4.3.1 Statistical validation

A multivariate statistical analysis was performed with SAS (SAS Institute Software) using the time to relapse as dependent variable and all the post-surgical available parameters (Gleason Score, pathological stage, type of surgery, margins and lymph nodes metastasis) plus the alpha coefficient, calculated as described in previous subsection, as independent variables.

The multivariate analysis was repeated by excluding the non statistically significant parameters. Moreover, the value of the alpha parameter calculated on the whole PSA series (from 4 to 17 values measured during the follow up, i.e. from 2 to 10 years after surgery) was compared with that estimated using only the first three PSA values recorded after biochemical failure.

Wherever possible, data were inserted into the model as continuous variables, so that the corresponding parameter estimate computed by the model was the actual slope of the straight line on which the data were fitted. The sign of the parameter estimate determined whether the dependent variable increased (+) or decreased (-) with respect to the independent one, the absolute value determined the steepness of the slope. Qualitative variable as well as quantitative ones which were not continuously distributed in their range were inserted as dummy variables.

In the box plots representation for the correlation between T and alpha classes the lozenge corresponds to the median value, the lines extending vertically from the boxes (whiskers) indicate the variability outside the upper and lower quartiles. Box plots representation is preferred because it is non-parametric, i.e. no assumption of the underlying statistical distribution to variation in samples of the population is required. The spacings between the different parts of the box indicate the degree of dispersion (spread) and skewness in the data, and show outliers.

### 4.3.2 Results and conclusions

Fig 1 shows the experimental data from patients with (left) and without (right) adjuvant ADT. It is apparent that the ratio  $r$  computed using the PSA values increases according to the proposed universal law for both populations.

As far as patients without any adjuvant therapy are concerned, the statistical analysis (see the table in Fig. 4.2) shows that the time to relapse is independent from any clinical information apart from the first post-operative PSA value and the alpha parameter (see Fig. 4.3). In particular, alpha is predictive of the range of the time of relapse, being the smaller alpha values predicting the longer free from disease time. In our sample, we found that  $0 < \alpha < 0.01$  implies  $T > 48$  months with a probability of 75%,  $0.01 < \alpha < 0.05$  implies  $T < 48$  months with a probability of

52.72%,  $0.05 < \alpha < 0.09$  implies  $T < 48$  months with a probability of 86.6% and  $T < 24$  in the 48.89% of cases,  $\alpha > 0.09$  implies  $T < 24$  months with a probability of 90%.

Contrary to retrospective studies, waiting for a long PSA series to make a prevision is impractical and ethically questionable in clinical practice: since PSA dosage is usually prescribed every 3 – 6 months, waiting for sixth-eighth PSA values makes predicting early recurrences senseless.

We excluded the cases in which surgery was not effective (e.g. the first PSA after prostatectomy is  $>0.2$  ng/ml and probably a local recurrence or a metastasis is present) or the relapse is very aggressive ( $T < 12$  months). We found that the same results about the predictability of alpha are valid using alpha\_3, i.e. the parameter alpha calculated only on the third PSA value of the series (no average), see Fig.4.4. In fact, the third PSA dosage after RP could be a useful marker of the trend of the PSA. The first two values, indeed, could be less representative (equal to zero or little oscillating due to other factors, e.g. inflammations).

We can conclude that a careful collection of PSA values would be valuable and overcome traditional clinical parameters (such as pGS, pathological staging, etc.) to predict the timing of the tumor recurrence development.

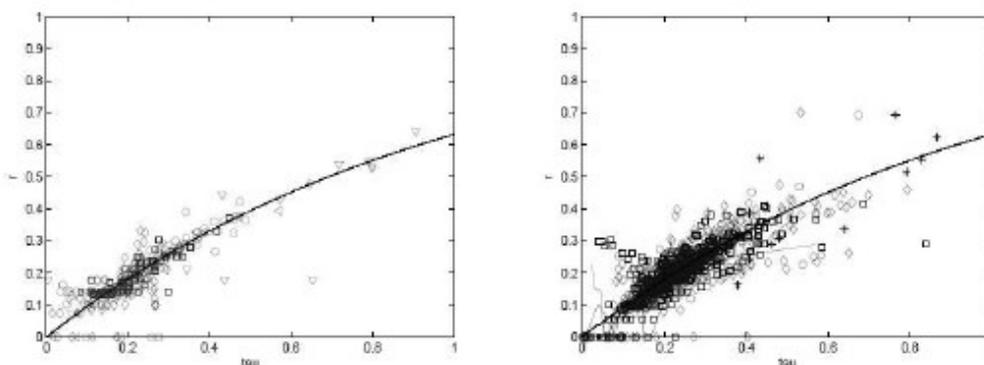


Figure 1:

Figure 4.1: The experimental data from patients who underwent ADT (right) or not (left).

	DF	Type III SS	Mean Square	F Value	Pr > F
<b>pGS</b>	6	6.03467373	1.00577896	1.90	0.0832
<b>PathologicalStage</b>	5	2.65039010	0.53007802	1.00	0.4190
<b>Pn</b>	1	0.14423792	0.14423792	0.27	0.6025
<b>PostSurgeryPSA</b>	1	29.93258121	29.93258121	56.47	<.0001
<b>Surgery technique</b>	2	1.86431463	0.93215732	1.76	0.1751
<b>PositiveMargins</b>	1	0.13879602	0.13879602	0.26	0.6094
<b>AlphaClass</b>	4	86.80204514	21.70051129	40.94	<.0001

Figure 2:

Figure 4.2: SAS output of the statistical analysis. The statistically significant parameters, in relation with the time of recurrence, are the alpha and the post-surgical PSA, that is strictly related to the alpha.

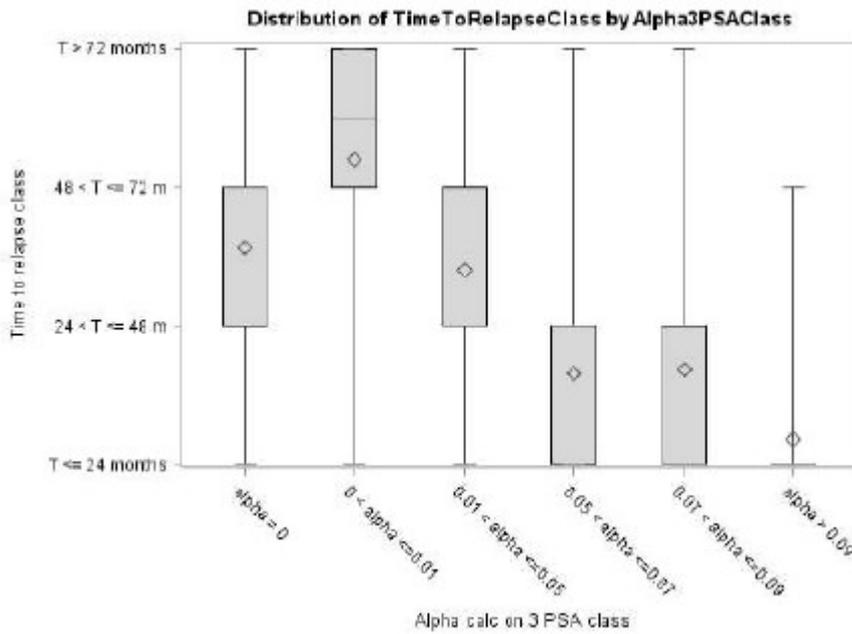


Figure 4.3: Distribution of the time to relapse by alpha\_3: the value of alpha\_3 can be used to distinguish the time of the relapse in most of the cases.

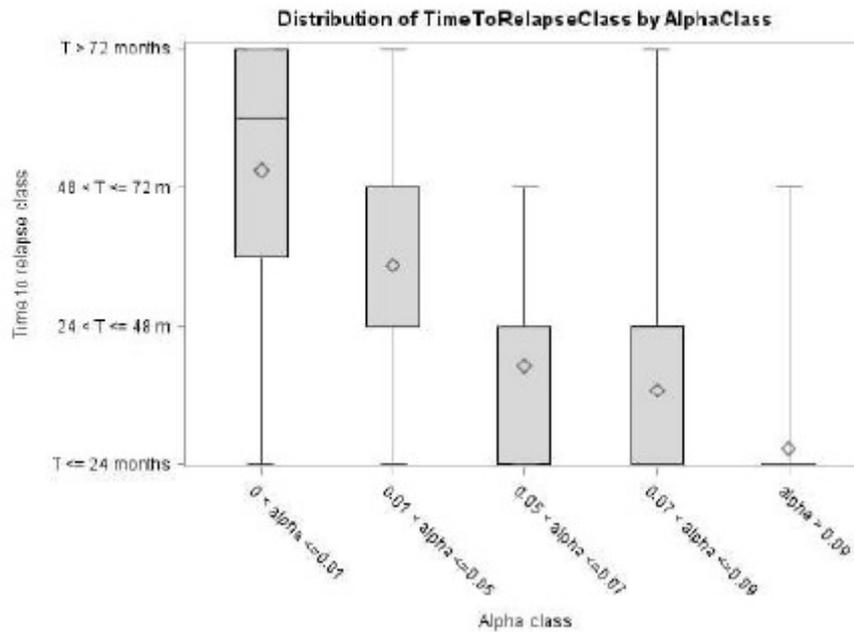


Figure 4.4: Distribution of the time to relapse by alpha: the alpha value can be used to distinguish the time to relapse.

## 5 Predictive (hypo-) models for PCa recurrence after radiotherapy

### 5.1 PSA is only one of the parameters related to the recurrence probability

Contrary to surgery, RT does not abolish the production of PSA by the prostate gland. A very much complex situation occurs. Following RT the PSA reduction may be very slow, and the lowest value, called ‘nadir’ may be detected after two years. Moreover, transient increases of PSA, called ‘bounces’, may be monitored especially after brachithery.

The definition of biochemical relapse is therefore much more critical, and different definitions have been given.

The first definition of biochemical relapse was proposed by ASTRO about 20 years ago. The panel found also a consensus about the significance of the nadir, the definition of rising PSA and biochemical relapse, and aimed to draw guidelines for the use of PSA as an indicator of success or of relapse following Radiation Therapy [18].

The main elements of the consensus were:

- 1) three consecutive increases in PSA are required to consider the patient in biochemical relapse after radiotherapy;
- 2) the date of relapse should be placed in the middle of time interval between the nadir PSA and the first of the three consecutive increases (“backdating” relapse);
- 3) PSA should be required every 3-4 months for the first two years of follow-up and every six months later, the results of patients with 1 or 2 increases must be kept separate from those with standard relapse definition of 3 increases, and the period of median follow-up to publish a study must be at least of 24 months.

Unfortunately, these recommendations were almost never met and adopted in the clinical practice, as well as one of the final considerations of the Consensus, that biochemical relapse does not justify the immediate recourse to salvage therapies.

A Consensus Conference sponsored by ASTRO and Radiation Therapy Oncology Group took place in Phoenix, Arizona, on January 21<sup>st</sup>, 2005, to revise the ASTRO definition.

The conclusions of the Phoenix Consensus Conference [19] are as follows:

- 1) Biochemical relapse after radiation therapy, with or without hormone therapy, occurs when PSA is equal or exceeds the value of the sum of PSA nadir plus 2 ng/ml;
- 2) the date of relapse is contextual to this value, then “at call” and not “backdated”;
- 3) the definition does not apply to patients treated with cryotherapy or salvage radiotherapy;

4) the median follow-up of the published studies must always be longer than two years compared to control rates reported (e.g. with a follow-up of 5 years can be reported control rates at 3 years).

Finally, such a complex clinical scenario makes it practically impossible to make a strong correlation between PSA and tumor (re)growth, as it would be needed by a mathematical (hypo)model.

Consequently, predictive models in the case of RT can only rely on clinical and histological parameters from previous exams, which can be arranged as ‘nomograms’.

## ***5.2 PCa epidemiology, histological and clinical parameters resumed in nomograms***

Multiple clinical nomograms have been developed in the past 25 years to reliably classify patients in different risk-classes according to various outcomes (see Appendix 8).

Between those used in RT we will describe the Roach formula, D’Amico risk classification, NCCN guidelines and Dimonte Doubling-Time model.

*Roach formula* for nodal involvement is a mathematical translation of Partin Tables published by M. Roach III in 1994 [20] and used mainly by radiation oncologists. The risk of positive nodes (N+) was calculated using the following equation:

$$N+ = 2/3(PSA) + (GS-6) \times 10$$

where PSA and GS are the pre-treatment prostate specific antigen and Gleason Score respectively.

In the original paper, patients were split according to a risk of nodal involvement < 15% (low-risk group) or patients with a calculated risk as > or = 15% (high-risk group). The observed incidence of positive nodes was 6% and 40% among the low and high-risk groups respectively ( $p < 0.001$ ).

Based on these data, many radiotherapists worldwide have adopted a policy of omitting prophylactic whole pelvic irradiation in patients identified as low-risk according to Roach formula.

*D’Amico risk classification* is a simple clinical nomogram combining pre-treatment PSA, clinical stage and biopsy GS in a categorical 3 x 3 table. It was first published by A.V. D’Amico in 1998 [21] and extensively used in the following decade by radiation oncologists and surgeons for treatment choice between Radical Prostatectomy, EBRT or interstitial brachytherapy for clinically localized prostate cancer (D’Amico nomogram is shown below in the Appendix 8).

*NCCN guidelines*, continually updated by the National Comprehensive Cancer Network [5], are a more complex classification system derived from D’Amico risk nomogram. Its main differences with D’Amico classifier are the inclusion of cT2c tumors in intermediate risk-class (and so not high-risk one) and to split patients up to 6 different risk-classes: very-low risk, low risk, intermediate risk, high risk, very-high risk and metastatic disease.

NCCN guidelines are up-to-date the most used algorithm for treatment choice by physicians worldwide. In particular, Version 2, 2014 [4], is the following:

*Clinically localized disease*

1. Very-low risk group:

- a) T1c
- b) Gleason Score  $\leq 6$
- c) PSA  $< 10$  ng/ml
- d) Fewer than 3 prostate positive biopsy cores,  $\leq 50\%$  cancer in each
- e) PSA density  $< 0.15$  ng/ml/g

2. Low risk group:

- a) T1-T2a
- b) Gleason Score  $\leq 6$
- c) PSA  $< 10$  ng/ml

3. Intermediate risk group:

- a) T2b-T2c OR
- b) Gleason Score 7 OR
- c) PSA 10-20 ng/ml

4. High risk group:

- a) T3a OR
- b) Gleason Score 8-10 OR
- c) PSA  $> 20$  ng/ml

*Locally advanced disease*

5. Very-high risk:

T3b-T4

6. *Metastatic disease:*

Any T, N1

Any T, Any N, M1

Patients with multiple risk factors may be shifted into the next highest risk group. Besides, when either PSA or Gleason Score or Staging is not available, grouping could be determined by the combination of the available parameters.

*Dimonte Doubling-Time model*, published by G. Dimonte in 2010 [22], is based on a cell kinetics model developed to describe the evolution of prostate cancer from diagnosis to prostate cancer specific death. It is able to stratify patients for Prostate Cancer Specific Survival according to post-treatment PSA Doubling-Time [22].

To describe the observed clinical progression, the model must postulate three prostate cancer cell populations that are: 1 – local to the prostate and sensitive to hormones, 2 – regional and hormone sensitive, and 3 – systemic and hormone resistant.

A set of coupled first-order differential equations describes the exponential growth of a prostate cancer tumor as well as its transformation from a local to systemic disease. The time dependence of the solutions is scaled to PSA Doubling Time (PSADT) because it characterizes the tumor growth for the individual.

### **5.3 The Candiolo nomogram**

The study was based on a large database of 2493 patients affected by prostate cancer and treated with EBRT as primary treatment belonging to the EUREKA-2 retrospective multi-centric database, including 3776 cases of radio-treated prostate cancer cases in North-West Italy between 1997 and 2012, approved by FPO-IRCCS Cancer Center of Candiolo Ethical Committee in July 2013 and amended in November 2014.

In particular, from the whole database were excluded 1283 patients without complete information regarding established pre-treatment factors (PSA, clinical-radiologic stage and bGS) and the number of total and positive biopsy cores.

In all cases, staging evaluation included anamnesis, physical exam with Digital Rectal Examination (DRE), serum PSA and a trans-rectal ultrasound (TRUS) guided needle biopsy of the prostate with GS histologic grading. Radiological examinations (abdominal CT, endo-coil or pelvic MRI and bone scan) were performed according to the patient risk-class, to the physician's opinion and to the available hospital facilities.

All patients were treated with curative 3-Dimensional Conformal EBRT (3D-CRT) or Intensity Modulated Radiation Therapy (IMRT). The fractionation schedules to prostate-GTV (Gross Tumor Volume) varied between traditional fractionation of 1.8-2 Gy per fraction to moderate hypofractionation of 2.5-2.7 Gy per fraction; all doses were normalized to Equivalent Dose at 2 Gy per fraction (ED2Gy) using a mean  $\alpha/\beta$  of 2.5 Gy for prostate cancer (according to literature  $\alpha/\beta$  ratio for prostate cancer ranges between 1.5 and 5.7 Gy [23-25]). Treatment consisted of radiotherapy alone or radiotherapy combined with ADT in 38% and 62% of the cases, respectively.

Median follow-up of the 2493 patients was 50 months. Standard follow-up included PSA and DRE every 3-months for 2 years, every 6-months until the fifth year and annually thereafter.

During the follow-up 453 patients (18%) had a biochemical relapse, 249 (10%) relapsed clinically, 138 (5.5%) had distant metastases, and 233 (9%) died, 72 of these (3% of the total) because of prostate cancer. Time 0 was defined as the last day of EBRT for all patients and PSA failure

according to Phoenix consensus definition (i.e. a rise by 2 ng/mL or more above the nadir PSA[26]).

Clinical relapse was defined as a recurrence in the prostate bed, regional lymph nodes or distant metastasis shown by radiologic examinations (bone scan, choline-PET-CT, MRI, CT, ultrasound) or by physical examination or by biopsy. Systemic relapse was defined as a distant metastasis, including bone or other visceral organs, shown by radiologic examinations or by physical examination. Prostate cancer specific mortality was defined as death because of prostate cancer, checked by a physician through patients' case history reports, cancer regional registries and, if necessary, phone calls to the patient or to a close relative or General Practitioner of him (if the patient was dead).

### 5.3.1 Statistical analysis

A Cox regression time to PSA failure analysis was performed in univariate and multivariate settings, evaluating the predictive ability of age, pre-treatment PSA, clinical-radiological stage, GS and %PC; besides, the regression algorithm was adjusted for RT dose (as a continuous variable) and combined therapy (RT+ADT or RT alone, as a dichotomous variable). The assumptions of the Cox model were tested and met.

All variables were evaluated as categorical variables: age  $\geq 70$  years or age  $< 70$  years; PSA  $< 7$  ng/ml, 7-15 ng/ml, or  $> 15$  ng/ml; clinical-radiologic stage cT1, cT2 or cT3-cT4; bGS  $\leq 6$ , 3+4, 4+3, 8 or 9-10; %PC 1-20%, 21-50%, 51-80% or 81-100%. Effects cell coding (i.e. 1 or 0 or -1 coding) was applied to the stratified variables, in order to calculate the relative Hazard Ratios (HRs) of the multivariate analysis compared to the outcome of the mean of the cohort. The accuracy of the model was checked with bootstrapping statistics (2493 patients, resampling with 1000 cases each replication for a total of 10,000 replications).

A 360-cells-table was built by multiplying the HRs of the subgroups for all the variables' combinations; the following combined HRs were classified into 5 different risk classes for biochemical relapse: very-low-risk for HRs 0.17-0.30; low-risk for HRs 0.31-0.55; intermediate-risk for HRs 0.56-1.20; high-risk for HRs 1.21-2.40; and very-high-risk for HRs 2.41-6.60.

In addition, an equivalent nomogram was built multiplying 100 by the beta coefficients associated to the HRs, normalizing each reference value of the variables to 0 points (i.e. age  $\geq 70$ , PSA  $< 7$  ng/ml, stage cT1, bGS  $\leq 6$  and %PC 1-20%), and summing up the values of the 5 prognostic factors into a scale ranging in between 0 and 363 total points, corresponding to the previously defined 5 risk classes.

Kaplan-Meier survival curves for bPFS, divided according to our 5-risk-classes and to D'Amico risk classes (for comparison), were graphed, overall and paired log-rank tests were performed and Concordance Indexes calculated. In addition, D'Amico risk classification and our 5-groups classification tool were further analyzed for cPFS, sPFS and PCSS and their prediction performances compared.

All statistical analyses were performed with Stata SE 13.1 Software (<sup>®</sup>StataCorp, Texas, USA).

### 5.3.2 Results

All variables evaluated, i.e. age, pre-treatment PSA, clinical-radiologic stage, bGS and %PC are highly significant predictors of biochemical relapse in both univariate and multivariate Cox models: all Ps are lower than 0.001 except for age, whose values are  $P=0.001$  and  $P=0.019$  in univariate and multivariate analyses, respectively (**Table 5.1**).

The 360-cells-table combining all the possible combinations of the stratified parameters clearly shows a strong trend, going from very-low risk (in blue) on the upper-left corner to very-high-risk (in red) in the lower-right corner; in between can be noticed low-risk (in green), intermediate-risk (in yellow) and high-risk (in orange, see **Table 5.2** and **Table 5.3**).

Very-low-risk group includes 529 patients (21%), low-risk 770 (31%), intermediate risk 696 (28%), high-risk 329 (13%) and very-high risk 169 (7%); full data on patients' distribution according to model parameters are illustrated in **Table 5.4**. Besides, the related Candiolo nomogram is displayed in **Figure 5.1**.

In addition, **Table 5.5** resumes yearly (until 10 years of follow-up) bPFS, cPFS, sPFS and PCSS for the five-classes of the Candiolo classifier. In particular, bPFS ranges at 5-yy between 94% (very-low-risk) and 43% (very-high-risk) and at 10-yy between 90% and 14%; cPFS varies at 5-yy between 97% and 62% and at 10-yy between 94% and 38%; sPFS ranges at 5-yy between 100% and 71% and at 10-yy between 100% and 56%; PCSS ranges at 5-yy between 100% and 86% and at 10-yy between 100% and 70%.

The Candiolo classifier differs significantly from the D'Amico risk classification.

- pre-treatment PSA: D'Amico advises the cut-offs of 10 and 20 ng/ml, while our optimal boundaries are slightly lower: 7 and 15 ng/ml.
- bGS: we refined the traditional coarse classification in 3-classes ( $\leq 6$ , 7 or  $\geq 8$ ) dividing patients according to a more precise 5-classes layering, in particular discerning 3+4 versus 4+3 patterns and 8 versus 9-10 ones.
- Staging: the D'amico clinical staging, splitting patients according to their cT2 stage ( $\leq$ cT2a, cT2b or  $\geq$ cT2c) was replaced by a clinical and radiological staging taking advantage of radiologic information and dividing patients according to more objective criteria: cT1 microscopic cancer, cT2 macroscopic but intra-prostatic cancer, cT3-4 macroscopic and extra-prostatic adenocarcinoma.

In addition, we added %PC and age in the prognostic algorithm.

In the early 2000s, D'Amico clearly proved the independent value of positive prostate biopsies in predicting biochemical outcome after radical prostatectomy or after EBRT[28]. The information about tumor extension at biopsy was clinically significant to better classify intermediate-risk patients; in both studies patients were stratified in three classes: %PC<34%, 34-50%, %PC>50%.

The statistical significance of the variable age was the lowest between the selected prognostic factors of biochemical recurrence and failed the internal validation with bootstrapping.

However, its relevance may be much more powerful for late end-points like sPFS and PCSS, and so this could be one of the determinant factors of the higher concordance indexes for these end-points by the Candiolo classifier (around 80%). In fact, two main reasons can justify the worse prognosis of younger patients: intrinsic biologic differences of prostate cancer and variations in patient's hormonal status at different ages, and a greater likelihood of experiencing progression or death since they are less likely to die of competing comorbidity conditions.

Our five classes are statistically different at paired comparisons for bPFS, cPFS, sPFS, while for PCSS three main groups may be identified: very-low and low-risk together (summing up to 52% of the overall cohort) with almost no dead at 10 years, intermediate and high-risk with a specific mortality at 10-yy around 10% and very-high-risk alone with a 10-yy mortality of 30%.

The mean prostate cancer specific survival in our cohort at 5 and 10 years are 97.5% and 92.5%, respectively. These high values may be justified by the intense therapy, performed both as a first-line treatment (62% of the cases where treated with the combination RT+ADT) and/or as a salvage or palliative therapy after biochemical and/or clinical recurrence (ADT alone 82%, chemotherapy 3%, local therapies i.e. surgery, RT, HIFU and cryotherapy 6%, ADT plus local therapy 3%, none 6%).

The main strengths of our study are: the large numerosity of our sample (2493 patients), widely distributed according to risk factors combinations (e.g. PSA from <1 to >100 ng/ml), and with complete staging and treatment information; the integration of five significant prognostic factors according to all their possible combinations; the five-risk classification instead of three-risk-classes with wider prognostic differences; the Proportional Hazard assumption of the Cox model fulfilled (see **Figure 5.3**); the internal validation with bootstrapping performed; the nomogram, developed with bPFS as setting end-point, was successfully applied to other three following outcomes, i.e. cPFS, sPFS and PCSS.

The study limitations are mainly related to its retrospective and multi-centric nature, and to the absence of an external validation. In particular, the biopsy schemes adopted by each center were heterogeneous and there wasn't any central pathologic review (even if by a decade Piedmont Oncology Web fosters pathology quality assessment on prostate cancer through guidelines and periodic meetings [29,30]).

Table 5.1 – Univariate and multivariate Cox regression (time to PSA failure) and bootstrapping analysis

**Table 2 – Univariate and multivariate Cox regression (time to PSA failure) and bootstrapping analysis.**

Variables	Sub-groups	Univariate Analysis		Multivariate Analysis		Bootstrapping
		HR	P value	HR	P value	P value
Age	≥ 70 yy	0.85	0.001	0.89	0.019	0.16
	< 70 yy	1.18		1.12		
PSA	< 7	0.52	< 0.001	0.63	< 0.001	< 0.001
	7-15	0.88		0.96		
	> 15	2.17		1.65		
Staging	cT1	0.49	< 0.001	0.77	< 0.001	0.031
	cT2	0.95		0.93		
	cT3-4	2.17		1.40		
bGS	≤ 6	0.47	< 0.001	0.59	< 0.001	0.012
	3+4	0.77		0.83		
	4+3	0.99		0.95		
	8	1.31		1.26		
	9-10	2.13		1.70		
% Positive Cores	1-20%	0.48	< 0.001	0.67	< 0.001	0.008
	21-50%	0.76		0.89		
	51-80%	1.22		1.11		
	81-100%	2.26		1.50		
RT Dose	Continuous	0.88 / Gy	< 0.001	0.89 / Gy	< 0.001	0.001
Therapy schedule	RT alone	0.81	< 0.001	1.25	< 0.001	0.028
	RT & ADT	1.24		0.80		

Table 5.2 – Candiolo classifier table: very-low risk in blue, low-risk in green, intermediate-risk in yellow, high-risk in orange, very-high risk in red

Table 3 – Candiolo classifier table: very-low-risk blue, low-risk green, intermediate-risk yellow, high-risk orange, very-high-risk red.

Initial PSA	Positive Cores %	Age	GS ≤ 6			GS 3 + 4			GS 4 + 3			GS 8			GS 9 - 10			
			cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4	
PSA < 7	1-20%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
	21-50%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
	51-80%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
81-100%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	
	<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	
PSA 7-15	1-20%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
	21-50%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
	51-80%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
81-100%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	
	<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	
PSA > 15	1-20%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
	21-50%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
	51-80%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
		<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
81-100%	≥70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	
	<70	Blue	Blue	Green	Blue	Green	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	

Table 5.3 – Candiolo classifier table with Hazard Ratio combination

SUPPLEMENTARY MATERIAL

Table 1-5 – Candiolo classifier table with Hazard Ratio combinations.

Initial PSA	Positive Cores %	Age	GS ≤ 6			GS 3 + 4			GS 4 + 3			GS 8			GS 9 - 10				
			0,59	0,59	0,59	0,83	0,83	0,83	0,95	0,95	0,95	1,26	1,26	1,26	1,70	1,70	1,70		
			cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4		
PSA < 7	1-20%	0,63 ≥70	0,67	0,93	1,4	0,77	0,93	1,4	0,77	0,93	1,4	0,77	0,93	1,4	0,77	0,93	1,4		
		0,63 <70	0,67	1,12	0,21	0,26	0,39	0,30	0,36	0,55	0,35	0,42	0,63	0,46	0,53	0,83	0,62	0,75	1,13
	21-50%	0,63 ≥70	0,89	0,89	0,23	0,27	0,41	0,32	0,39	0,58	0,37	0,44	0,66	0,48	0,58	0,88	0,65	0,79	1,19
		0,63 <70	0,89	1,12	0,29	0,34	0,52	0,40	0,48	0,73	0,46	0,55	0,84	0,61	0,74	1,11	0,82	0,99	1,49
	51-80%	0,63 ≥70	1,11	0,89	0,28	0,34	0,51	0,40	0,48	0,72	0,46	0,55	0,83	0,60	0,73	1,10	0,81	0,98	1,48
		0,63 <70	1,11	1,12	0,36	0,43	0,65	0,50	0,60	0,91	0,57	0,69	1,04	0,76	0,92	1,38	1,03	1,24	1,86
81-100%	0,63 ≥70	1,5	0,89	0,38	0,46	0,69	0,54	0,65	0,98	0,62	0,74	1,12	0,82	0,99	1,48	1,10	1,33	2,00	
	0,63 <70	1,5	1,12	0,48	0,58	0,87	0,68	0,82	1,23	0,77	0,94	1,41	1,03	1,24	1,87	1,39	1,67	2,32	
PSA 7-15	1-20%	0,96 ≥70	0,67	0,89	0,26	0,31	0,47	0,37	0,44	0,67	0,42	0,51	0,76	0,56	0,67	1,01	0,75	0,91	1,36
		0,96 <70	0,67	1,12	0,33	0,40	0,60	0,46	0,56	0,84	0,53	0,64	0,96	0,70	0,84	1,27	0,94	1,14	1,71
	21-50%	0,96 ≥70	0,89	0,89	0,35	0,42	0,63	0,49	0,59	0,88	0,56	0,67	1,01	0,74	0,89	1,34	1,00	1,20	1,81
		0,96 <70	0,89	1,12	0,43	0,53	0,79	0,61	0,74	1,11	0,70	0,85	1,27	0,93	1,12	1,69	1,25	1,51	2,28
	51-80%	0,96 ≥70	1,11	0,89	0,43	0,52	0,78	0,61	0,73	1,10	0,69	0,84	1,26	0,92	1,11	1,67	1,24	1,50	2,26
		0,96 <70	1,11	1,12	0,54	0,65	0,99	0,76	0,92	1,39	0,87	1,05	1,59	1,16	1,40	2,11	1,56	1,89	2,84
81-100%	0,96 ≥70	1,5	0,89	0,58	0,70	1,06	0,82	0,99	1,49	0,94	1,13	1,70	1,24	1,50	2,26	1,68	2,03	3,05	
	0,96 <70	1,5	1,12	0,73	0,88	1,33	1,03	1,24	1,87	1,18	1,42	2,15	1,56	1,89	2,84	2,11	2,55	3,84	
PSA > 15	1-20%	1,65 ≥70	0,67	0,89	0,45	0,54	0,81	0,63	0,76	1,14	0,72	0,87	1,31	0,95	1,15	1,74	1,29	1,56	2,34
		1,65 <70	0,67	1,12	0,56	0,68	1,02	0,79	0,96	1,44	0,91	1,09	1,65	1,20	1,45	2,18	1,62	1,96	2,95
	21-50%	1,65 ≥70	0,89	0,89	0,59	0,72	1,08	0,84	1,01	1,52	0,96	1,15	1,74	1,27	1,53	2,31	1,71	2,07	3,11
		1,65 <70	0,89	1,12	0,75	0,90	1,36	1,05	1,27	1,91	1,20	1,45	2,19	1,60	1,93	2,90	2,15	2,60	3,91
	51-80%	1,65 ≥70	1,11	0,89	0,74	0,89	1,35	1,04	1,26	1,89	1,19	1,44	2,17	1,58	1,91	2,88	2,13	2,58	3,88
		1,65 <70	1,11	1,12	0,93	1,13	1,69	1,31	1,58	2,38	1,50	1,81	2,73	1,99	2,40	3,62	2,89	3,24	4,88
81-100%	1,65 ≥70	1,5	0,89	1,00	1,21	1,82	1,41	1,70	2,56	1,61	1,95	2,93	2,14	2,58	3,89	2,88	3,48	5,24	
	1,65 <70	1,5	1,12	1,26	1,52	2,29	1,77	2,14	3,22	2,03	2,45	3,69	2,89	3,25	4,89	3,69	4,38	6,60	

Table 5.4 – Candiolo Classifier Table with complete patients’ distribution

Table 2-5 – Candiolo classifier table with complete patients’ distribution.

Initial PSA	Positive Cores %	Age	GS ≤ 6			GS 3 + 4			GS 4 + 3			GS 8			GS 9 - 10		
			cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4	cT1	cT2	cT3-4
PSA < 7	1-20%	≥70	86	49	4	19	15	2	5	9	0	5	3	0	0	0	0
		<70	50	39	5	5	5	0	0	2	0	0	0	0	0	0	0
	21-50%	≥70	64	91	6	22	48	5	11	20	1	5	22	2	1	5	6
		<70	24	39	4	7	16	1	2	7	0	2	4	2	0	2	1
	51-80%	≥70	8	13	2	3	13	2	5	4	1	3	7	1	1	3	1
		<70	5	12	0	2	3	2	1	2	1	2	1	1	0	3	3
81-100%	≥70	4	6	1	2	9	2	0	3	0	3	2	0	1	4	4	
	<70	1	11	0	1	1	2	1	0	0	0	4	0	0	2	0	
PSA 7-15	1-20%	≥70	74	53	1	18	14	0	6	9	1	9	6	1	1	3	0
		<70	32	18	6	1	4	0	2	2	1	0	1	0	0	0	0
	21-50%	≥70	55	108	10	20	64	6	10	28	3	10	22	5	5	8	1
		<70	24	40	4	4	22	2	4	13	3	1	10	3	0	2	3
	51-80%	≥70	12	23	6	5	24	2	0	18	2	2	8	3	1	6	2
		<70	4	10	1	1	11	3	0	3	2	1	1	2	0	3	2
81-100%	≥70	5	16	0	3	13	6	4	7	1	2	13	3	1	9	6	
	<70	3	8	1	2	3	2	0	3	0	0	1	3	0	4	3	
PSA > 15	1-20%	≥70	22	14	0	5	7	1	2	7	0	4	4	1	0	0	0
		<70	6	8	1	1	2	1	2	0	0	1	1	1	0	0	0
	21-50%	≥70	14	25	5	5	13	5	3	14	2	4	16	4	1	8	3
		<70	4	8	2	2	13	3	1	3	1	1	8	6	0	1	0
	51-80%	≥70	1	15	2	3	13	5	1	3	4	2	13	6	1	11	0
		<70	0	9	2	0	12	2	0	7	3	1	2	0	0	2	2
81-100%	≥70	1	15	2	0	15	6	1	12	3	0	13	8	0	10	14	
	<70	0	6	2	0	13	7	0	10	9	0	13	6	1	5	12	

Table 5.5 – Life tables: bPFS, cPFS and PCSS for the five-classes of the Candiolo Classifier

Table 4 – Life tables: bPFS, cPFS, sPFS and PCSS for the five-classes of the Candiolo classifier.

Candiolo Risk Class	biochemical PFS – Follow-up (years)									
	1	2	3	4	5 yy	6	7	8	9	10 yy
Very-Low	99%	99%	96%	95%	94%	92%	90%	90%	90%	90%
Low	99%	97%	94%	90%	85%	84%	82%	80%	76%	74%
Intermediate	98%	94%	90%	83%	80%	74%	69%	65%	63%	60%
High	95%	85%	78%	71%	67%	60%	54%	52%	47%	43%
Very-High	83%	72%	60%	51%	43%	36%	35%	31%	23%	14%
Candiolo Risk Class	clinical PFS – Follow-up (years)									
	1	2	3	4	5 yy	6	7	8	9	10 yy
Very-Low	100%	100%	99%	99%	97%	97%	96%	94%	94%	94%
Low	99%	99%	98%	96%	94%	92%	91%	89%	88%	88%
Intermediate	99%	97%	95%	93%	92%	89%	87%	85%	85%	82%
High	98%	93%	89%	84%	79%	76%	68%	66%	63%	55%
Very-High	89%	81%	74%	68%	62%	56%	53%	51%	42%	38%
Candiolo Risk Class	systemic PFS – Follow-up (years)									
	1	2	3	4	5 yy	6	7	8	9	10 yy
Very-Low	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Low	100%	100%	99%	98%	97%	97%	96%	95%	94%	94%
Intermediate	100%	98%	97%	95%	94%	93%	92%	91%	91%	91%
High	99%	95%	93%	90%	89%	88%	82%	82%	80%	80%
Very-High	90%	82%	77%	73%	71%	68%	66%	64%	60%	56%
Candiolo Risk Class	PCSS – Follow-up (years)									
	1	2	3	4	5 yy	6	7	8	9	10 yy
Very-Low	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Low	100%	100%	99%	99%	99%	99%	98%	98%	98%	98%
Intermediate	100%	100%	99%	98%	98%	98%	96%	95%	95%	92%
High	100%	99%	99%	96%	95%	93%	93%	92%	89%	89%
Very-High	99%	95%	92%	87%	86%	80%	80%	76%	70%	70%

Figure 5.1 – The Candiolo nomogram.

**Figure 1 – Candiolo nomogram.**

**Points:**

**bGS** ≤6 0 pt, bGS=3+4 35 pt, bGS=4+3 48 pt, bGS=8 76 pt, bGS=9-10 106 pt;

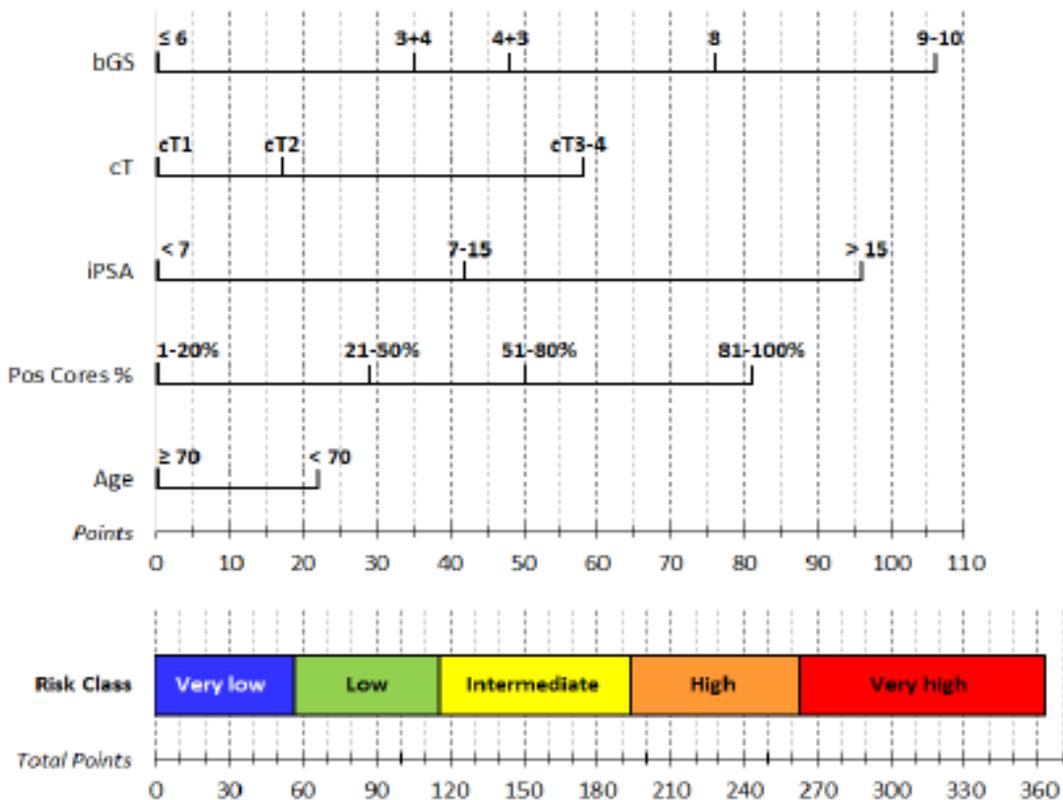
**cT** cT1 0 pt, cT2 17 pt, cT3-4 58 pt;

**PSA** <7 0 pt, PSA7-15 42 pt, PSA>15 96 pt;

**%PC** 1-20% 0 pt, 21-50% 29 pt, 51-80% 50 pt, 81-100% 81 pt;

**age** ≥70 0 pt, age<70 22 pt;

**very-low risk** 0-56 pt, **low risk** 57-116 pt, **intermediate risk** 117-193 pt, **high risk** 194-262 pt, **very-high risk** 263-363 points.



## 6 (Hypo-) models for evaluating resistance induction by hormonal therapies

### 6.1 Introduction

Normally, the prostate cancer cells are hormone-sensitive cells. They produce PSA and they need a hormone to stimulate growth. There is a very small group, however, which is not hormone-sensitive but hormone-resistant [31]. This type of cells does not need hormones to duplicate but its growth potential is very low respect to the hormone-sensitive ones.

Nowadays, hormone therapies are very common to contrast the growing of hormone-sensitive tumors, like breast and prostate cancer. In this last case the growth of hormone-resistant cells will finally induce an almost uncontrollable increase after an initial reduction of the tumor volume [31]. Any realistic model should therefore take into account the appearance of therapy-induced cell mutations (or phenotypic modifications [32]).

The key question in this context is how strong can the interplay of the different cell populations be. Since they are part of the same organism, a "minimal" hypothesis states that they share the same overall energetic resources. It is therefore reasonable to assume that the total tumor carrying capacity is limited, and the growth of both cell populations is constrained [33,34].

We investigated an asymmetrical two-cell-populations model, identifying its equilibria. Their stability or instability expresses the successful cure or the fatal evolution of the tumor. We identify the parameter conditions ensuring the stable configuration, i.e. the situation where the tumor stops growing.

### 6.2 Two populations model

We assume that the two populations respond to treatments differently. In particular is proved [31] that there is an androgen dependent (AD) cell population, very sensitive to the hormone therapy while an androgen independent (AI) population less sensitive or not sensitive at all to it.

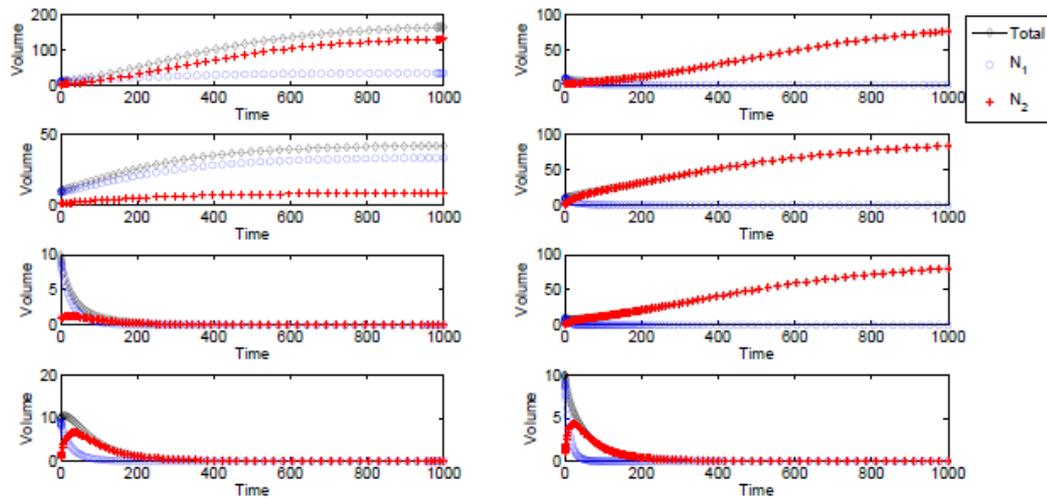
The system is:

$$\begin{cases} \frac{dN_1}{dt} = c_1(t)N_1 - d_1(t)N_1 \\ \frac{dN_2}{dt} = c_2(t)N_2 - d_2(t)N_2 + \epsilon N_2(t) \end{cases}$$

where  $d_1(t)$  and  $d_2(t)$  are the treatment kill rates on the populations  $N_1$  and  $N_2$  respectively and  $\epsilon$  is a measure of the metabolic rate increment in response to particularly favourable growth conditions. It also expresses an additional growth rate for the second population. In principle,  $d_1$  and  $d_2$  could be functions of time to account for different times of treatments and one- or multi-shot therapies.

The mathematical analysis of the system allows us to predict the behaviour of the two populations, knowing the parameters values. Moreover, we are able to know threshold values for the kill rates in order to eradicate one or both populations. For example, we can say that in the Gompertzian model the AD can be considered eradicated when  $d_1 > 10 r_1$ . The best scenario (death of both populations) is not attained so easily, since  $d_2 \gg \epsilon + r_2$ .

### 6.3



Mutation and treatment in  $U_1$  case. The parameter reference values in all frames, unless otherwise noted, are  $r_1 = 0.005, r_2 = 0.003, m = 0.005, d_1 = 0.005, d_2 = 0.003$ . Top row left: all parameters are at the low reference values entailing coexistence of the two populations. Top row right: high kill rate of the first population ( $d_1 = 0.05$ ), but the second one survives; Second row left: high kill rate of the second population ( $d_2 = 0.03$ ), the first one prevails; Second row right: high mutation rate ( $m = 0.05$ ), only the second one thrives; Third row left: high kill rates ( $d_1 = 0.05, d_2 = 0.03$ ), both populations are eradicated; Third row right: high kill rate of the first population and high mutation rate ( $d_1 = 0.05, m = 0.05$ ), the first population decreases rapidly; Bottom row left: high kill rate of the second population and high mutation rate ( $d_2 = 0.03, m = 0.05$ ), both populations vanish; Bottom row right: high kill and high mutation rates ( $d_1 = 0.05, d_2 = 0.03, m = 0.05$ ), both populations disappear.

### Results and discussion

The approach of the "Phenomenological Universalities" allows a satisfactory investigation of the growth of an asymmetrical two-population cancer. Different interactions were studied, corresponding to different clinical scenarios, i.e. the growth of both populations constrained by a fixed total carrying capacity, the response to treatments, the occurrence of spontaneous or induced mutations.

We applied the Gompertzian and West functions to model the growth of the cells populations in a manageable and realistic way. They have been successfully validated on various tumor scenarios, finding analytical solutions whenever possible. Numerical simulations assessed the effectiveness and role of the model parameters in the remaining cases.

Our two-clones model confirms that effective ADT can reduce the AD cell population although the eradication of the AI cells is much more critical. This happens in the presence of spontaneous mutations and, even worse, when mutations are induced or promoted by therapies.

The West growth assumption represents an optimal model for the simulations of the tumor development and response to therapies. This happens because of the biological significance of the growth parameter, related to the cellular metabolic rate and duplication energy of each specific cell population. Moreover, a clinical application is provided.

This work can be used in other contexts, in particular in the study of the three main cancers scenario. In fact, we challenged our model against lung (Non-small cell lung, NSCL) human cancer data (from [35]). NSCL cancer is known to be composed by two different phenotypes, i.e. hypoxic cells in the core and well oxygenated cells in the outer ring. Oxygenated cells exhibit a large growth rate but a low resistance to chemo therapy. Hypoxic cells, on the contrary, have a low growth rate but a larger resistance to chemo therapy. In the database we chose, growth and kill rates, initial and final volumes

after therapy as well as growth and drug kill rates were available. We tested our model using two scenarios:  $m=0.35$  (bad response to the treatment) and  $m=0.09$  (good response to the treatment).

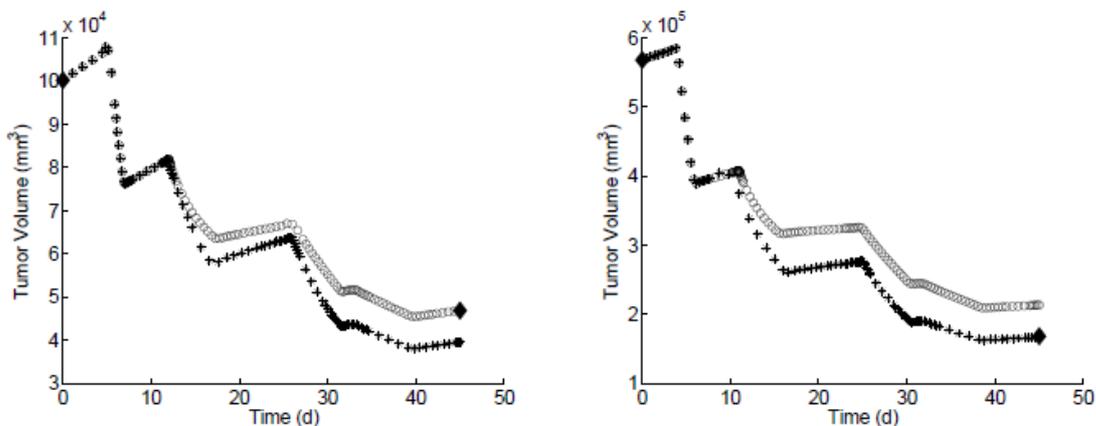


Figure : Simulations of cells mutation and for the patient response to treatment in the Gompertzian case. Black rhombus are the measure of the real tumor volumes pre ( $t = 0$ ) and after ( $t = 45$ ) chemo-therapy. The circles represent the simulations performed with  $m = 0.35$ , the stars those obtained with  $m = 0.09$ . The patient on the left shows a bad response to treatment, since the final tumor size is related to the mutation rate  $m = 0.35$ ; the patient on the right shows a good response to treatment since the final tumor size is instead related to  $m = 0.09$ .

Unfortunately, since only the final tumor volume is available and not the two single populations (large dot in Figure), we cannot evaluate the actual value of the mutation rates.

In conclusion, the model (see [36] for further details) could be useful to simulate different treatment scenarios and, provided a careful validation of the parameter values is carried on extensive clinical database, it may help in the preliminary estimation of the expected effectiveness of different therapeutic approaches.

## 7 Present and next future task

### 7.1 Upgrade of the data collection EUREKA1 and EUREKA2

In order to implement larger clinical database and to extend their follow up time, an amendment to the previously submitted EUREKA1 and EUREKA2 studies has been requested (and obtained) by the Ethical Committee of FPO-IRCCS Center of Candiolo (see Appendix 5). The EUREKA-1 database will be extended to provisionally 3700 cases (including new clinical data), the EUREKA-2 database will be extended to 5300 cases (including new centres and new techniques).

A subcohort of 90 patients who underwent adjuvant post Radiotherapy continuous or intermittent ADT is available for further modelling validation.

In September 2015 a monocentric collection of DICOM images has begun at FPO-IRCCS Cancer Center of Candiolo (EUREKA-2). The study is focusing on 99 patients treated homogeneously at the Radiation Oncology Division with IMRT-IGRT technique. Staging and re-staging data will be available too.

In particular, data collected will include:

- a) Radiation therapy images plus DVH data: CT planning, Contouring data, DVH in graph format (and possibly RT plan, RT dose, DVH in .txt format according to human resources committed to the project), all 99 patients available;
- b) mp-MRI (multi-parametric-MRI) data plus medical reports: T2-weighted morphologic scans, DWI and DCE functional sequences, staging exams (69 available) plus re-staging examinations, if available;
- c) Choline-PET-CT data plus medical reports: [18F]-FluoroCholine-PET plus low-dose CT scans, staging exams (28 available) plus re-staging examinations, if available.

Data will be stored on the central servers at FPO-IRCCS Cancer Center of Candiolo and UNITO. Data will be pseudonymized for research purposes; thereafter, the data will be visualized and analyzed through RADIANT software (for radiologic images) and DICOM-PILER software (for RT treatment plans). Data analyses will include: evaluation of target coverage and its relationship with oncologic outcomes; assessment of organs-at-risk DVHs and correlation with observed acute and late toxicities; evaluation of the value of extensive radiologic staging with mp-MRI and choline-PET-CT and its influence on RT treatment planning and outcomes; longitudinal estimate of the response to treatment comparing pre- and post-RT MRI (e.g. ADC maps) or pre- and post-PET images (e.g. SUVmax).

Data collection is foreseen to end within March 2016 and data analysis within June 2016.

#### 7.1.1 Implementation of a sub-cohort of patients treated by brachithrapy

In addition, a data collection on prostate cancer patients treated with radical brachytherapy (EUREKA-3 study) at Sassari Hospital, Radiotherapy University Division (Director G. Meloni, Responsible M.F. Dedola, operator A. Carnevale) in partnership with the Urology Division, Alghero Hospital (Director A. Tedde) and the Medical Physics Unit, Sassari Hospital (Director P.G. Marini, operator M. Tamponi).

The total number of patients treated with Iodine-125 permanent seeds by 2002 is approximately 200. Besides, in the latter hundred patients (by 2008), the procedure has been technically implemented with the employment of the “Target Scan” device, a 3D stereotactic sonography system that improves the precision of image-guided seeds implant.

The collection of a brachytherapy cohort may be in particular useful to compare outcomes and collateral effects with EBRT and surgery, and to externally validate RT nomograms in the risk-classes fit for brachytherapy, i.e. low and very-low risk cases.

## **7.1.2 Modeling of salvage therapies:**

### **7.1.2.1 Model approach to conventional and non-conventional salvage radiotherapy treatment schedules**

Tumor volume data pre and post primary Radiotherapy will be used to implement and validate the model. This is based on the estimation of the radio-biological parameters of the tumor. It will evaluate the therapeutic effectiveness of conventional and non-conventional (e.g. hypo and hyper-fractionated) treatment schedules in order to predict the best approach for different risk classes. The model will improve the performance of a previous work already published by the team (see Radiation Research).

### **7.1.2.2 Model approach to ADT and CT salvage therapy treatment schedules**

The hypomodel will predict the tumor response to treatments, according to the collected data. We will take into account also the available knowledge about the adaptations/mutations occurring in the PCa cells undergoing ADT or other CT. Vaccination strategies will be evaluated as well.

## 8 Exploitation and dissemination issues for extended clinical validation

### 8.1 *Towards a 'computational-based recurrence predictor' prototype*

The hypo models presented in Ch. 4, 5 and 6, together with the ongoing model activities on therapies, could be the base for a 'computational-based recurrence-predictor' SW (Uro-Angel).

A prototype could be tailored and proposed to the EUREKA1 and EUREKA2 clinical networks.

#### 8.1.1 **Testing the compliance of the clinicians networks towards the prototype**

The first test of the uro-angel prototype would be the assessment of its compliance among the clinicians involved in the trial. Different versions would be released based on the observations of those who test it, according to how it is manageable, easy to be filled with clinical data, easy to be run, robust, safe and user-friendly.

#### 8.1.2 **Predisposing the ethical and technical frames for further clinical validation**

After a good compliance is gained, clinical validation would require the design of a perspective multicenter clinical trial to be submitted to the Ethical Committee; and also a technical frame to guarantee proper use and privacy issues for all the Centres participating to the study.

### 8.2 *Personalized medicine approach: prevention and rehabilitation of surgery collateral effects*

Following Radical Prostatectomy a temporary impairment of the urogenital functions is normally suffered. Incontinence problems normally disappears within a few months, but it may continue to occur. This worsen the patient's quality of life, unless specific therapeutic strategy is performed.

Rehabilitation of the pelvic pavement is one of the best approach. The basic idea is to integrate two different approaches: a user-friendly visual interface and a more rigorous control by the clinician using quantitative parameters.

As concerns the first approach, we want to create a set of 3D animations using free tools like Unity3D and Blender, then to use them as 'serious games' usable by the patients. These SW allow planning of personalized 'at home' training sessions, with or without the active surveillance of the clinician.

As concerns the second approach, the improvements of the patient should be checked at each step with technological tools, like motion capture or ultrasound analysis.

## 9 Conclusion

In conclusion the past, present and future activity related to the CHIC infrastructure applied to Prostate Cancer can be sketched in the following picture. This shows the different scales at which the hypomodels already developed or in progress, as well as the possible exploitations, can integrate the CLINICAL HYPERMODEL for Prostate Cancer:

hypomodels	Radical prostatectomized Patients (RP)	Radical Radiotreated (RRT) patients
MOLECULAR LEVEL (circulating bio marker PSA)	<b>X</b> PSA-HYPO	
CELLULAR LEVEL	<b>X</b> ADT-HYPO	<b>X</b> ADT-HYPO IMAGE-HYPO
ORGAN-TISSUE LEVEL	<b>X</b> SALVAGE RT-HYPO	<b>X</b> (‘Candiolo’ nomogram-HYPO) SALVAGE ADT- CT HYPO
WHOLE BODY LEVEL- PERSONALIZED	<b>X</b> rehabilitation	

Green= done

Yellow= work in progress

Red= exploitation

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## Appendix 1 - Inclusion and Exclusion criteria – EUREKA-1 Study

### APPENDIX 1 – Inclusion and Exclusion criteria - EUREKA-1 Study

**Inclusion criteria:**

- 1) histologic diagnosis of adenocarcinoma of the prostate;
- 2) Radical Prostatectomy +/- lymphadenectomy as first-line treatment, performed within the period January 1<sup>st</sup>, to December 31<sup>st</sup>, 2012;
- 3) At least two of the following three pre-treatment parameters: PSA, staging, Gleason Score;
- 4) At least two years of follow-up available;
- 5) Case history available for clinical data collection.

**Exclusion criteria:**

- 1) Histologic diagnosis other than adenocarcinoma of the prostate;
- 2) Radical Prostatectomy performed before January 1<sup>st</sup>, 1999 or after December 31<sup>st</sup>, 2012;
- 3) Radical Prostatectomy not performed as first-line treatment;
- 4) Not consecutive cases.

## Appendix 2 – Inclusion and Exclusion criteria – EUREKA-2 Study

### APPENDIX 2 – Inclusion and Exclusion criteria - EUREKA-2 Study

**Inclusion criteria:**

- 1) Histologic diagnosis of adenocarcinoma of the prostate;
- 2) Radical Radiotherapy as first-line treatment, performed with conformational technique within the period January 1<sup>st</sup>, 1997 to December 31<sup>st</sup>, 2012;
- 3) At least two of the following three pre-treatment parameters: PSA, staging, Gleason Score;
- 4) At least two years of follow-up available;
- 5) Case history available for clinical data collection.

**Exclusion criteria:**

- 1) Histologic diagnosis other than adenocarcinoma of the prostate;
- 2) Radical Radiotherapy performed before January 1<sup>st</sup>, 1997 or after December 31<sup>st</sup>, 2012;
- 3) Radical Radiotherapy not performed as first-line treatment, or not performed with conformational treatment;

## Appendix 3 - EUREKA-1 study - firstly approval by FPO-IRCCS Ethical Committee



FONDAZIONE PIEMONTESE  
PER LA RICERCA SUL CANCRO  
ONLUS

**COMITATO ETICO**

Candiolo, 9 luglio 2013

Dott. Pietro Gabriele  
Responsabile U.O.A.  
di Radioterapia  
IRCC CANDIOLO

**OGGETTO: Valutazione** “Studio osservazionale retrospettivo multicentrico per la definizione di modelli prognostici sul cancro prostatico in pazienti operati – European multicentric Retrospective Study evaluating prognostic factors on prostate K (CAncer) in prostatectomysed patients “ – EUREKA-1

Le comunico che nella seduta dell’8 luglio 2013 il Comitato Etico dell’Istituto, esaminato e discusso il protocollo in oggetto, ha espresso **parere favorevole**.

Si fa presente che:  
La sperimentazione va condotta nel rispetto della normativa vigente e dei regolamenti dell’istituzione presso la quale deve essere svolta, secondo i principi etici fissati nella Dichiarazione di Helsinki e della buona pratica clinica (D.M.S. 15.07.1997).  
Si fa, inoltre, presente che lo sperimentatore ha l’obbligo di comunicare la data di inizio della sperimentazione.  
Il responsabile della sperimentazione ha l’obbligo di riferire immediatamente al Comitato Etico indipendente:

- le eventuali variazioni del protocollo;
- tutte le reazioni avverse da farmaci soprattutto se serie ed inattese;

segue



Indirizzo Strada Provinciale 142, Km 3.95, 10060 Candiolo (TO) T 011.9933.380 - F 011.9933.389 c/c Postale 410100 - C. F. 97519070011 UniCredit  
IBAN IT 64 T 02008 01154 000008780163 - Intesa Sanpaolo IBAN IT 07 0 03069 01000 100000516980 - web www.fprconlus.it - e-mail fprc@fprconlus.it

CONSIGLIO DIRETTIVO: **Presidente** Allegra Agnelli. **Vice Presidenti** Carlo Acutis, Maria Vaccari Scassa. **Consigliere Delegato** Giampiero Gabotto. **Consiglieri** Marco Boglione, Bruno Ceretto, Paolo Maria Comoglio, Giuseppe Della Porta, Gianluca Ferrero, Gianluigi Gabetti, Giuseppe Gilardi, Maria Elena Giraud Rayneri, Eugenio Lancellotta, ~~Anna Maria Marocco~~, Aldo Ottavis, Carlo Pacciani, Lodovico Passerin d’Entrèves, Patrizia Re Rebaudengo Sandretto, Silvio Saffirio, Piero Sierra. **Direttore Scientifico dell’Istituto di Candiolo** Paolo Maria Comoglio. **REVISORI DEI CONTI: Presidente** Giacomo Zunino. **Componenti** Mario Boldi, Lionello Jona Celesia.

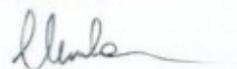
Riconoscimento della Regione Piemonte: D.G.R. 22/07/1986 n° 3-6673 - Iscrizione anagrafe Onlus prot. n. 9882440 del 19/06/1998

2)

- ogni nuova informazione che possa incidere negativamente sulla sicurezza dei soggetti e sulla conduzione dello studio.
- Lo sperimentatore deve dare comunicazione sulla conduzione dello studio al Comitato Etico ogni anno e, comunicare senza ritardo, l'esito della sperimentazione.

Con i migliori saluti

Il Presidente  
(Carlo Luda di Cortemiglia)



Prot. C.E. 0171/2013

brl/CLC

## Appendix 4 - EUREKA-2 study - firstly approval by FPO-IRCCS Ethical Committee



FONDAZIONE PIEMONTESE  
PER LA RICERCA SUL CANCRO  
ONLUS

**COMITATO ETICO**

Candiolo, 9 luglio 2013

Dott. Pietro Gabriele  
Responsabile U.O.A.  
di Radioterapia  
IRCC CANDIOLO

**OGGETTO:** Valutazione “Studio osservazionale retrospettivo multicentrico per la definizione di modelli prognostici sul cancro prostatico in pazienti radio trattati” EUREKA-2

Le comunico che nella seduta dell’8 luglio 2013 il Comitato Etico dell’Istituto, esaminato e discusso il protocollo in oggetto, ha espresso **parere favorevole**.

Si fa presente che:  
La sperimentazione va condotta nel rispetto della normativa vigente e dei regolamenti dell’istituzione presso la quale deve essere svolta, secondo i principi etici fissati nella Dichiarazione di Helsinki e della buona pratica clinica (D.M.S. 15.07.1997).  
Si fa, inoltre, presente che lo sperimentatore ha l’obbligo di comunicare la data di inizio della sperimentazione.  
Il responsabile della sperimentazione ha l’obbligo di riferire immediatamente al Comitato Etico indipendente:

- le eventuali variazioni del protocollo;
- tutte le reazioni avverse da farmaci soprattutto se serie ed inattese;

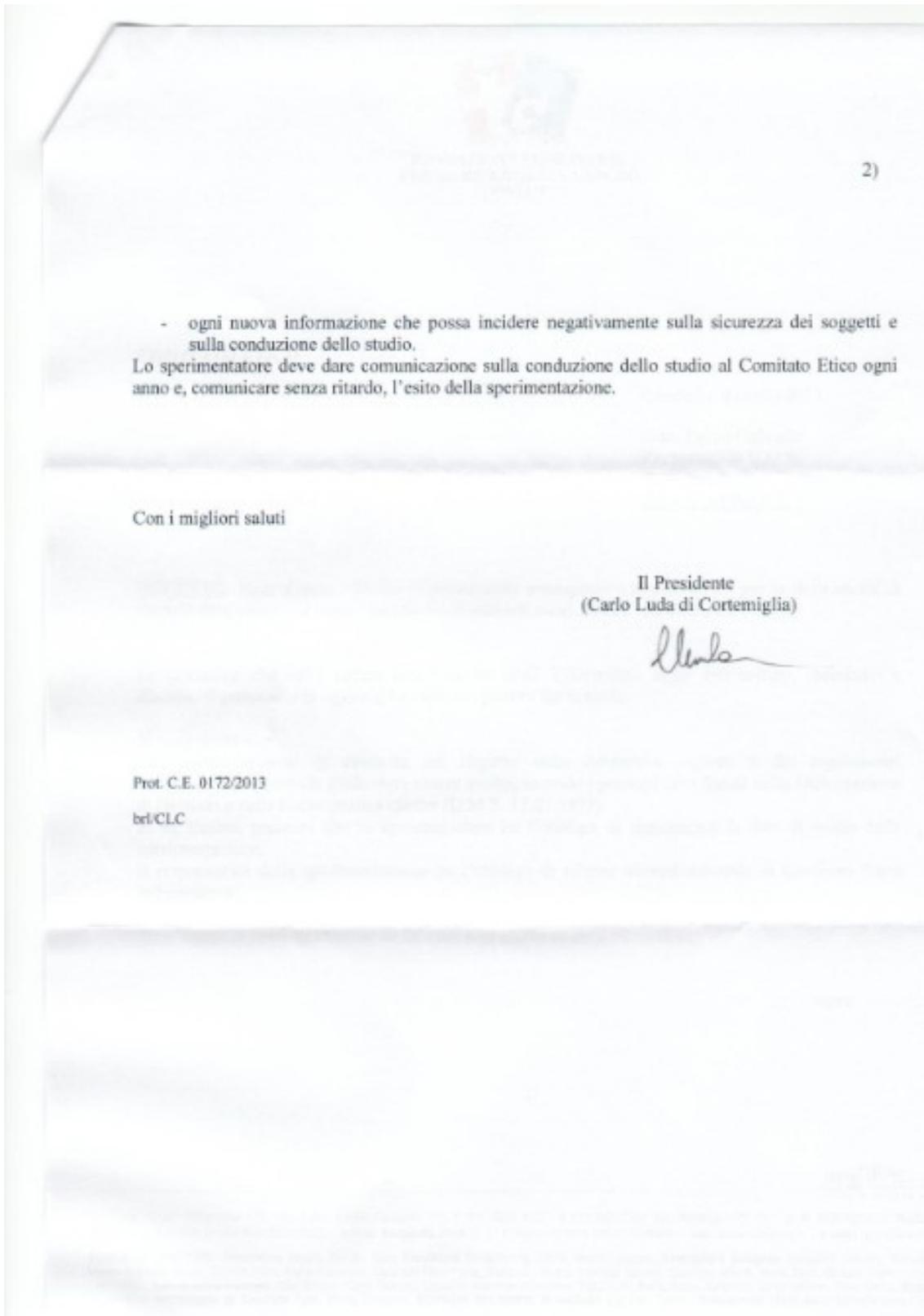
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IBAN IT 64 T 02008 01154 000008780163 - Intesa Sanpaolo IBAN IT 07 O 03069 01000 100000516980 - web www.fprconlus.it - e-mail fprc@fprconlus.it

**CONSIGLIO DIRETTIVO:** Presidente Allegra Agnelli. Vice Presidenti Carlo Acutis, Maria Vaccari Scassa. Consigliere Delegato Giampiero Gabotto. Consiglieri Marco Boglione, Bruno Ceretto, Paolo Maria Comoglio, Giuseppe Della Porta, Gianluca Ferrero, Gianluigi Gabetti, Giuseppe Gilardi, Maria Elena Giraudo Rayneri, Eugenio Lancellotta, ~~Antonio Maria Mancuso~~, Aldo Ottavio, Carlo Pacciani, Lodovico Passerin d’Entrèves, Patrizia Re Rebaudengo Sandretto, Silvio Saffirio, Piero Sierra. Direttore Scientifico dell’Istituto di Candiolo Paolo Maria Comoglio. **REVISORI DEI CONTI:** Presidente Giacomo Zunino. Componenti Mario Boldi, Lionello Jona Celesia.

Riconoscimento della Regione Piemonte: D.G.R. 22/07/1986 n° 3-6673 - Iscrizione anagrafe Onlus prot. n. 9882440 del 19/06/1998



## Appendix 5 - Amendedament of EUREKA-2 as versions 2.0 in November 12th, 2014

Azzivo 088/14

 IRCCS - Istituto di Ricovero e Cura a Carattere Scientifico

FONDAZIONE DEL PIEMONTE PER L'ONCOLOGIA  
protocollo n° 438  
del 4.12.2014

COMITATO ETICO IRCCS  
Candiolo

Candiolo, 20 novembre 2014

Allo Sperimentatore Principale  
Dottor Pietro Gabriele

Al Direttore Sanitario FPO  
Dr. Piero Fenu

e p.c. Al Direttore Generale  
Fondazione del Piemonte per l'Oncologia  
IRCCS CANDIOLO (TO)

Pratica n. 27/C-2013  
Protocollo n.417/2014  
**Seduta del 12 novembre 2014**

Delibera del Comitato Etico IRCCS di Candiolo istituito con:  
Prov. 42 del 24/9/2013 del Direttore Generale FPO avviso pubblico per selezione componenti  
Prov. 53 del 5/11/2013 del Direttore Generale FPO per nomina Componenti  
Prot. 3326 del 3/12/2013 del Direttore Generale FPO per richiesta iscrizione Registro Reg.  
Comitati Etici

PROTOCOLLO: "Studio osservazionale a coorte storica multicentrico per la definizione di modelli prognostici sul cancro prostatico in pazienti radiotrattati – EUROpean multicentric historic cohort study Evaluating prognostic factors on prostate K (cAncer) in radiotreated patients" - EUREKA 2  
PROMOTORE: Fondazione del Piemonte per l'Oncologia  
SEDE: Fondazione del Piemonte per l'Oncologia IRCCS di Candiolo – Direzione di Radioterapia  
RESPONSABILE: Dr. Pietro Gabriele

Si comunica che il Comitato Etico IRCCS di Candiolo ha espresso nella seduta del 12 novembre 2014 **PARERE FAVOREVOLE** all'emendamento sostanziale v.2.0 del 10.10.2014.

Sono stati valutati i seguenti documenti:  
01 - Lettera richiesta parere per valutazione emendamento al C.E.  
02 - Protocollo versione 2.0 del 10.10.2014 EUREKA-2 (versione track e clean)  
03 - Sinossi EUREKA-2 vers.2.0 del 10.10.2014

FONDAZIONE DEL PIEMONTE PER L'ONCOLOGIA  
Ente giuridico di diritto privato senza scopo di lucro - Presidio ospedaliero accreditato ex art. 43 L. 833/78  
Strada Provinciale 142, Km 3.95, 10060 Candiolo (TO) - T Centralino 011.9933.111 - C.F. 95596990010 - P.IVA 10202940010

 Fondazione  
del Piemonte  
per l'Oncologia



IRCCS - Istituto di Ricovero e Cura a Carattere Scientifico  
TUTTO DI CANDIOLO

04 – CV del P.I. dello studio  
06 - Scheda Informativa  
07 - Lettera richiesta esonero versamento oneri C.E.  
08 - Dichiarazione in merito alla natura no profit dello studio  
09 - Consenso informato e foglio informativo EUREKA-2 v. 2.0 del 10.10.2014  
10 - Scheda Riepilogativa EUREKA-2 per CE IRCCS  
11 Lettera al Medico Curante

Questo Comitato in merito alla sperimentazione in oggetto dovrà essere informato:

- Dell'Inizio della sperimentazione e della sua conclusione
- Del verificarsi durante la sua conduzione di sospette reazioni avverse gravi e inattese che potrebbero influire sulla sicurezza del paziente o sul proseguimento dello studio
- Di ogni successivo emendamento e modifica sostanziale del protocollo approvato.

Il responsabile dello studio dovrà inoltre far pervenire una relazione annuale sull'andamento dello stesso.

A conclusione dello studio il responsabile dovrà infine presentare una relazione sintetica sui risultati dello studio e comunicare gli estremi bibliografici di eventuali pubblicazioni prodotte.

Il Comitato Etico IRCCS ricorda altresì che dovrà essere garantito, da parte degli sperimentatori, il diritto alla diffusione e/o pubblicazione dei risultati, favorevoli e non favorevoli, nel rispetto delle disposizioni vigenti in materia di protezione dei dati sensibili e che non devono sussistere vincoli di diffusione e pubblicazione da parte del promotore.

In ogni successiva comunicazione dovrà essere indicato il numero di pratica assegnato a questa sperimentazione.

Cordiali saluti

Il Presidente  
Comitato Etico IRCCS di Candiolo  
- Carlo Luda di Cortemiglia

Prot. CE IRCCS 442/2014

FONDAZIONE DEL PIEMONTE PER L'ONCOLOGIA  
Ente giuridico di diritto privato senza scopo di lucro - Presidio ospedaliero accreditato ex art. 43 L. 833/78  
Strada Provinciale 142, Km 3.95, 10060 Candiolo (TO) - T Centralino 011.9933.111 - C.F. 95596990010 - P.IVA 10202940010



## Appendix 6 – Database input variables for EUREKA-1 Study

<b>APPENDIX 6 – Database input variables for EUREKA-1 Study</b>
<p><i>Some data in the database are mandatory and so they are marked with an asterisk ( * ); other data are to be considered as optional.</i></p>
<p><b><u>Medical history and diagnosis:</u></b></p> <ol style="list-style-type: none"> <li>1. Family history for prostate cancer (defined as one first-degree relative or two second degree relatives suffering from prostate K)</li> <li>2. Number of diagnostic biopsy proved prostate K (sooner or later)</li> <li>3. Gangway biopsy (transperineal or transrectal)</li> <li>4. Presence of acute or chronic inflammation in the biopsy</li> <li>5. Presence of precancerous biopsy</li> <li>6. Dosage diagnostic urinary PCA3</li> <li>7. * Number of frustules championships during diagnostic biopsy</li> <li>8. * Number of frustules positive for prostate adenocarcinoma</li> <li>9. Percentage of frustules most affected positive for adenocarcinoma of the prostate</li> <li>10. Positivity frustules of unilateral versus bilateral</li> <li>11. PSA-velocity preoperative</li> </ol>
<p><b><u>Staging:</u></b></p> <ol style="list-style-type: none"> <li>1. * Initial preoperative PSA (IPSA)</li> <li>2. * clinical staging of T</li> <li>3. * primary biopsy Gleason score</li> <li>4. * secondary biopsy Gleason score</li> <li>5. * biopsy Gleason score total</li> <li>6. free-PSA (fPSA) expressed in ng / mL</li> <li>7. free-PSA expressed as a% of total PSA</li> <li>8. pro-PSA expressed in ng / mL</li> <li>9. pro-PSA expressed as a% of total PSA</li> <li>10. Dosage of CrA preoperative serum in U / L</li> <li>11. * Execution and results of Bone scintigraphy for the detection of bone metastases</li> <li>12. Execution and results of CT pelvis for seeking extension locoregional</li> <li>13. Execution and results of MRI (with endorectal coil) to search for ECE</li> <li>14. Implementation and result-choline PET for the detection of lymph node metastasis and remote</li> </ol>
<p><b><u>Therapies:</u></b></p> <ol style="list-style-type: none"> <li>1. * Date PR</li> <li>2. * type of prostatectomy performed (ie retropubic radical prostatectomy traditional Nerve-sparing unilateral or bilateral nerve-sparing prostatectomy or laparoscopic or robotic prostatectomy)</li> <li>3. * Execution of lymphadenectomy</li> <li>* 4. Experience of the surgical center (running less than 50 procedures per year or execution of 50 or more interventions per year)</li> <li>5. * Execution of Hormonal Neo-Adjuvant</li> <li>6. Start and duration of the OT neo-adjuvant</li> <li>7. Type neoadjuvant hormone therapy drug</li> <li>8. * Execution of Adjuvant Radiotherapy</li> <li>* 9. Treatment Center RT</li> <li>10. Give the start and end adjuvant RT</li> <li>11. RT technique (2D versus 3D versus IMRT versus IGRT)</li> <li>12. RT Dose</li> <li>13. Execution RT on the pelvic lymph nodes</li> <li>14. * Execution of Hormonal Adjuvant</li> <li>15. Start and duration OT adjuvant</li> <li>16. Type adjuvant hormone therapy drug</li> <li>17. * Other therapies (HIFU, cryotherapy)</li> </ol>

**Pathological anatomy:**

1. \* primary pathological Gleason score
2. \* secondary pathological Gleason score
3. \* pathological Gleason score total
4. pathological Gleason score tertiary
5. \* pathological staging of T
6. \* State of surgical margins
7. \* State of extra-capsular invasion (ECE)
- 8 \* State of the Seminal vesicle
9. \* pN
10. Number of lymph nodes removed
11. Number of positive lymph nodes histopathology after surgery
12. Extracapsular extension / break nodal
13. State of perineural invasion
14. State of vascular invasion
15. Involvement of the prostatic
16. Presence of high grade PIN definitive pathological examination
17. Percentage of cells positive for Ki67 definitive pathological examination
18. Percentage of positive cells Chromogranin A (CrA) pathological examination definitive

**Follow up:**

1. \* Date of last follow-up
2. Presence of biochemical recurrence of disease (BCR)
3. \* If there is BCR, date of BCR
4. Presence of clinical relapse (CR)
5. \* If there is CR, date of CR
6. \* Site of clinical recurrence (prostate bed versus regional lymph node metastases versus bone metastases versus another organ to be specified site versus no clinical relapse assessable)
7. \* Death
8. \* Death from Prostate Cancer
9. \* Death from other causes
10. \* If death, date of death
11. \* PSA nadir postoperative (NPSA)
12. \* Type test used for dosing the NPSA (sensitive to the hundredth of ng / mL versus ultrasensitive to the thousandth of ng / mL)
13. \* Date of PSA nadir
14. \* Three doses of PSA after the NPSA with their dates of execution
15. Further dosages postoperative PSA with dates
16. Examinations FU (SOTB, CT, MRI with or without endorectal coil, PET-choline, take exams, PSA pre-exam date, OT in place or not)

**Toxicity:**

1. Presence of incontinence after surgery
2. Presence of impotence following surgery

**Appendix 7 - Database input variables for EUREKA-2 Study**

## APPENDIX 7 – Database input variables for EUREKA-2 Study

*Some data in the database are mandatory and so they are marked with an asterisk ( \* ); other data are to be considered as optional.*

### **Medical history and diagnosis:**

1. Family history for prostate cancer (defined as one first-degree relative or two second degree relatives suffering from prostate K)
2. Presence of acute or chronic inflammation in the diagnostic biopsy
3. Presence of precancerous in the diagnostic biopsy
4. Dosage of urinary PCA3
5. \* Number of frustules championships during diagnostic biopsy
6. \* Number of frustules positive for prostate adenocarcinoma
7. \* Percentage of frustule most affected positive for adenocarcinoma of the prostate
8. PSA-velocity pre-radiotherapy

### **Staging:**

1. \* initial PSA (IPSA)
2. PSA zenith (zPSA)
3. \* clinical-radiological staging of T
4. \* primary biopsy Gleason Score
5. \* secondary biopsy Gleason Score
6. \* biopsy total Gleason Score
7. free PSA (fPSA) expressed in ng / mL
8. free-PSA expressed as a% of total PSA
9. pro-PSA expressed in ng / mL
10. Pro-PSA expressed as% of total PSA
11. Dosage of CrA serum in U / L
12. \* Execution and eventual outcome of the Bone scintigraphy for the detection of bone metastases
13. Execution and eventual outcome of CT pelvis for seeking extension locomotive regional
14. \* Execution and eventual outcome of MRI (with endorectal coil)
15. State of extra-capsular invasion (ECE) to RM
16. State of seminal vesicle invasion MRI
17. \* cN (depending on MRI and / or on CT)
18. Execution and eventual outcome of the choline-PET for the detection of lymph node metastases and a distance

### **Therapies:**

1. \* Local experience in radiotherapy treatments (running less than 50 treatments per year or execution of 50 or more treatments per year)
2. \* Date of start and end of radiotherapy
3. \* Kind of radiotherapy technique performed (IMRT vs 3DCRT)
4. \* Using IGRT (yes / no)
5. \* Type of IGRT (Ultrasound, piling seeds radio-opaque, cone-beam CT, MVCT)
6. \* RT Dose to the PTV Primary tumor
7. \* fractionation RT
8. \* pelvic irradiation and, if performed, dose and fractionation
9. Presence of written preparation protocol of the bladder
10. Presence of written preparation protocol of the rectum
11. \* Average dose delivered to the bladder
12. \* Dose at V70 and V50 delivered to the rectum
13. \* Execution of neoadjuvant Hormonal Therapy (ADT)
14. \* Timing and duration neoadjuvant ADT
15. Type (# and dosage and administration) drug neoadjuvant ADT
16. \* Running concurrent ADT (yes/no)
17. Type (# and dosage and administration) concomitant ADT

18. \* Running Adjuvant ADT (yes/no)
  19. \* Timing and duration of adjuvant ADT
  20. Type (kind of drug # and dosage and administration) adjuvant ADT
  21. \* Other therapies (HIFU, cryotherapy) and their dates of execution
- Follow up:**
1. \* Date of last follow-up
  2. Presence of biochemical recurrence of disease (BCR)
  3. Rule of BCR (ASTRO vs Phoenix)
  4. \* If there is BCR, date of BCR
  5. Presence of clinical relapse (CR)
  6. \* If there is CR, date of CR
  7. \* Site of clinical recurrence (prostate bed versus regional lymph node metastases versus bone metastases versus another organ to be specified site versus no clinical relapse assessable)
  8. \* Death
  9. \* Death from Prostate Cancer
  10. \* Death from other causes
  11. \* If death, date of death
  12. \* PSA nadir postoperative (NPSA)
  13. \* Type test used for dosing the NPSA (sensitive to the hundredth of ng/mL versus ultrasensitive to the thousandth of ng/mL)
  14. \* Date of PSA nadir
  15. \* Five dosages of PSA after the NPSA with their dates of execution
  16. Further dosages postoperative PSA with dates
  17. Examinations FU (SOTB, CT, MRI with or without endorectal coil, PET-choline, PSA pre-exam date, current ADT or not)
- Toxicity:**
1. \* coughed acute (<6 months) Gastro-Intestinal (RTOG grading from 0 to 5)
  2. \* Toxicity late (> 6 months) Gastro-Intestinal (RTOG grading from 0 to 5)
  3. \* coughed acute (<6 months) Genito-Urinary (RTOG grading from 0 to 5)
  4. \* Toxicity late (> 6 months) Genito-Urinary (RTOG grading from 0 to 5)

## Appendix 8 – Some main nomograms used in Radiotherapy

**APPENDIX 8 – Some main nomograms used in Radiotherapy**

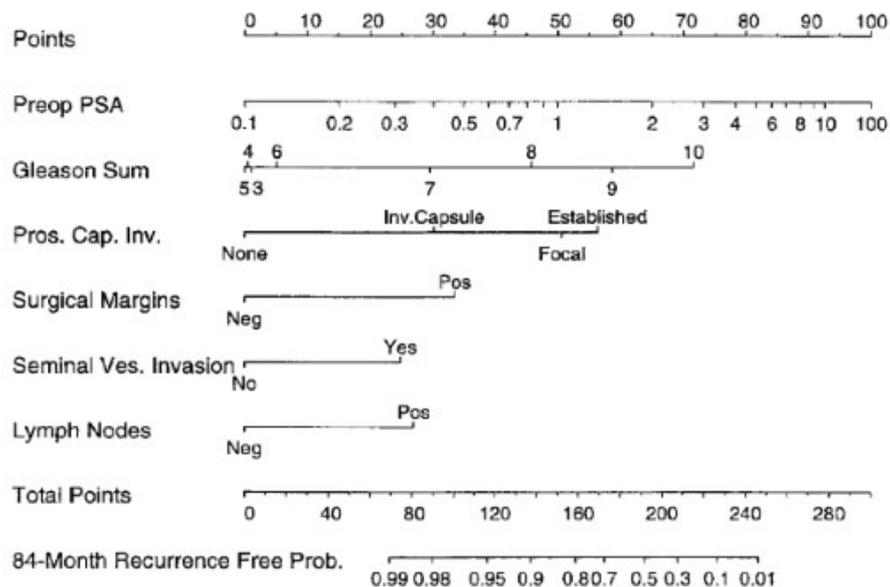
**1) D’Amico nomogram**

D’amico Risk Classification is a simple nomogram combining pre-treatment PSA, clinical stage and biopsy Gleason Score in a 3x3 table

Risk Class	PSA		Clinical Staging		Biopsy Gleason Score
Low	< 10	AND	T1-T2a	AND	≤ 6
Intermediate	10-20	OR	T2b	OR	7
High	> 20	OR	T2c-T3-T4	OR	≥ 8

**2) Kattan post- operative nomogram**

Based on 996 patients treated at The Methodist Hospital, Houston, TX, for predicting PSA biochemical recurrence after radical prostatectomy



**Instructions for Physician:** Locate the patient's PSA on the PSA axis. Draw a line straight upwards to the Points axis to determine how many points towards recurrence the patient receives for his PSA. Repeat this process for the other axes, each time drawing straight upward to the Points axis. Sum the points achieved for each predictor and locate this sum on the Total Points axis. Draw a line straight down to find the patient's probability of remaining recurrence free for 84 months assuming he does not die of another cause first.

**Instruction to Patient:** "Mr. X, if we had 100 men exactly like you, we would expect between <predicted percentage from nomogram - 10%> and <predicted percentage + 10%> to remain free of their disease at 7 years following radical prostatectomy, and recurrence after 7 years is very rare."

### 3) Partin Tables (2013 update)

Predicted probability (95% Confidence Interval) of pathological stage according to clinical stage (TNM), PSA level and biopsy Gleason score.

PSA	Pathological stage	Biopsy Gleason Score				
		6	3+4	4+3	8	9-10
<b>Clinical stage T1c (n = 4380)</b>						
0-2.5	OC (n = 289)	93 (91-95)	83 (78-87)	80 (74-85)	79 (72-85)	74 (61-83)
	EPE (n = 21)	7 (5-8)	15 (11-20)	17 (12-22)	18 (12-24)	20 (12-29)
	SV+ (n = 4)	0 (0-1)	2 (0-3)	3 (1-6)	3 (1-6)	5 (1-12)
	LN+ (n = 0)	0 (0-0)	0 (0-1)	0 (0-2)	0 (0-2)	2 (0-6)
2.6-4.0	OC (n = 751)	87 (85-89)	71 (67-75)	66 (60-71)	65 (57-72)	56 (44-67)
	EPE (n = 133)	12 (10-14)	25 (22-29)	27 (22-32)	28 (22-34)	29 (20-40)
	SV+ (n = 10)	0 (0-1)	2 (1-4)	4 (2-7)	4 (2-8)	7 (3-12)
	LN+ (n = 4)	0 (0-0)	1 (0-2)	3 (1-5)	3 (1-6)	8 (3-16)
4.1-6.0	OC (n = 1439)	84 (83-86)	66 (63-69)	60 (55-65)	59 (51-66)	50 (38-60)
	EPE (n = 371)	15 (13-16)	29 (26-33)	31 (26-36)	32 (25-38)	32 (23-42)
	SV+ (n = 37)	1 (0-1)	4 (2-5)	6 (4-9)	6 (4-10)	10 (5-16)
	LN+ (n = 11)	0 (0-0)	1 (0-2)	3 (2-5)	3 (1-6)	8 (4-15)
6.1-10.0	OC (n = 686)	80 (78-82)	59 (55-63)	53 (47-58)	52 (44-59)	42 (31-52)
	EPE (n = 226)	18 (16-20)	34 (30-38)	35 (30-40)	36 (29-43)	36 (26-46)
	SV+ (n = 36)	1 (1-2)	6 (4-8)	9 (6-13)	9 (5-14)	14 (8-21)
	LN+ (n = 8)	0 (0-0)	1 (0-2)	3 (1-5)	3 (1-6)	8 (4-14)
>10.0	OC (n = 191)	69 (64-74)	42 (36-48)	34 (28-40)	33 (26-40)	23 (15-32)
	EPE (n = 121)	27 (22-31)	42 (36-47)	28 (32-45)	39 (31-47)	33 (24-44)
	SV+ (n = 28)	3 (2-5)	13 (9-18)	20 (14-27)	20 (12-28)	25 (15-36)
	LN+ (n = 14)	0 (0-1)	3 (1-5)	8 (4-14)	8 (3-14)	18 (9-30)
<b>Clinical stage T2a (n = 897)</b>						
0-2.5	OC (n = 140)	90 (87-92)	76 (70-81)	72 (65-79)	71 (62-79)	65 (51-76)
	EPE (n = 23)	10 (7-13)	22 (17-28)	24 (17-30)	24 (18-33)	27 (18-39)
	SV+ (n = 1)	0 (0-1)	2 (0-4)	3 (1-7)	3 (1-7)	6 (1-13)
	LN+ (n = 1)	0 (0-0)	0 (0-1)	1 (0-4)	1 (0-3)	2 (0-9)
2.6-4.0	OC (n = 1399)	82 (78-84)	61 (56-66)	56 (48-62)	54 (46-63)	45 (33-56)
	EPE (n = 52)	18 (15-21)	34 (29-39)	35 (29-42)	36 (29-44)	36 (26-49)
	SV+ (n = 5)	1 (0-1)	3 (1-5)	5 (2-8)	5 (2-9)	7 (3-14)
	LN+ (n = 5)	0 (0-0)	1 (0-3)	4 (1-8)	4 (1-10)	11 (4-23)
4.1-6.0	OC (n = 183)	78 (74-81)	56 (51-60)	49 (43-56)	48 (40-56)	39 (28-50)
	EPE (n = 91)	21 (18-24)	38 (34-43)	39 (33-46)	40 (32-48)	39 (28-50)
	SV+ (n = 8)	1 (1-1)	4 (3-6)	7 (4-10)	7 (4-11)	10 (5-16)
	LN+ (n = 3)	0 (0-0)	2 (1-3)	4 (2-7)	4 (2-8)	11 (4-21)
6.1-10.0	OC (n = 104)	73 (68-77)	48 (43-54)	42 (36-49)	41 (33-50)	32 (23-43)
	EPE (n = 72)	26 (22-30)	44 (39-49)	44 (37-50)	45 (36-52)	43 (31-54)
	SV+ (n = 10)	1 (1-2)	6 (4-9)	10 (6-15)	10 (5-16)	14 (7-22)
	LN+ (n = 4)	0 (0-0)	1 (1-3)	4 (2-7)	4 (1-8)	10 (4-20)
>10.0	OC (n = 22)	60 (53-66)	32 (26-39)	25 (20-31)	24 (18-32)	16 (10-24)
	EPE (n = 22)	36 (30-42)	50 (43-56)	44 (36-53)	45 (35-55)	37 (25-49)
	SV+ (n = 10)	4 (2-6)	14 (8-20)	20 (12-29)	20 (11-30)	24 (13-38)
	LN+ (n = 2)	1 (0-2)	4 (2-7)	10 (4-18)	10 (4-20)	22 (10-37)
<b>Clinical stage T2b or T2c (n = 352)</b>						
0-2.5	OC (n = 26)	82 (76-87)	61 (52-70)	55 (45-66)	54 (44-66)	45 (32-60)
	EPE (n = 13)	17 (12-23)	33 (25-42)	34 (25-44)	35 (24-46)	35 (23-48)
	SV+ (n = 0)	1 (0-2)	5 (1-10)	8 (2-16)	8 (2-16)	13 (3-24)
	LN+ (n = 0)	0 (0-0)	1 (0-3)	2 (0-9)	3 (0-9)	7 (0-21)
2.6-4.0	OC (n = 27)	70 (63-75)	44 (37-51)	36 (29-44)	35 (27-44)	24 (16-35)
	EPE (n = 30)	28 (22-35)	46 (39-53)	43 (35-51)	44 (34-53)	37 (26-51)
	SV+ (n = 3)	2 (1-3)	6 (3-10)	10 (5-16)	10 (5-17)	13 (6-23)
	LN+ (n = 2)	1 (0-2)	4 (2-8)	11 (5-20)	11 (4-21)	25 (12-42)
4.1-6.0	OC (n = 52)	64 (58-70)	38 (32-44)	30 (24-37)	30 (22-37)	20 (13-29)
	EPE (n = 45)	32 (27-39)	49 (42-56)	45 (38-52)	46 (37-55)	38 (26-51)
	SV+ (n = 14)	2 (1-4)	9 (6-13)	14 (9-20)	13 (8-21)	17 (9-28)
	LN+ (n = 12)	1 (0-2)	4 (2-8)	11 (5-17)	11 (5-19)	24 (12-40)
6.1-10.0	OC (n = 25)	58 (50-65)	31 (25-37)	24 (19-31)	24 (18-31)	16 (10-23)
	EPE (n = 36)	38 (32-45)	52 (46-59)	47 (40-55)	48 (39-57)	40 (28-52)
	SV+ (n = 7)	4 (2-6)	12 (8-18)	19 (12-25)	18 (10-26)	23 (12-34)
	LN+ (n = 5)	1 (0-2)	4 (2-7)	10 (5-16)	10 (5-18)	22 (10-35)
>10.0	OC (n = 8)	42 (34-50)	17 (13-23)	12 (8-16)	11 (8-16)	6 (4-11)
	EPE (n = 21)	47 (39-55)	50 (41-59)	39 (30-49)	40 (28-51)	27 (18-40)
	SV+ (n = 18)	9 (5-14)	23 (15-33)	30 (20-41)	29 (18-42)	30 (17-45)
	LN+ (n = 8)	2 (0-4)	9 (4-16)	20 (10-31)	20 (9-32)	36 (20-53)

PSA, prostate-specific antigen; RP, radical prostatectomy; OC, organ confined; EPE, extraprostatic extension; SV+, seminal vesicle involvement; LN+, lymph node involvement.

## Appendix 9 – Abbreviations and acronyms

<i>SOA</i>	Service Oriented Architecture
<i>GPSM</i>	Gleason, PSA, Seminal Vesicle and Margin Status
<i>UGL</i>	Universal growth law
<i>PCa</i>	Prostate Cancer
<i>pGS</i>	pathological Gleason Score
<i>PSA</i>	Prostate Specific Antigen
<i>PUN</i>	Phenomenological Universalities
<i>AI</i>	Androgen Independent (cells)
<i>AD</i>	Androgen Dependent (cells)
<i>ADT</i>	Androgen Deprivation Therapy
<i>RP</i>	Radical Prostatectomy
<i>RR</i>	Radical Radiotherapy
<i>NSCL</i>	Non- small cell lung
<i>NB</i>	Nephroblastoma
<i>GB</i>	Glioblastoma
<i>RT</i>	Radiation Therapy
<i>CT</i>	Chemo Therapy
<i>EAU</i>	European Association of Urology
<i>NCCN</i>	National Comprehensive Cancer Network
<i>bGS</i>	bioptic Gleason Score
<i>EBRT</i>	External Beam Radiation Therapy
<i>PCR</i>	Piedmont Cancer Registry
<i>BCR</i>	BioChemical Recurrence

