Contents

1 Introduction

In most western industrialised countries, cancer and cardiovascular diseases constitute the main cause of death and assume more importance, as life expectancy in these societies increases. As illustrated in Figure 1, the most common and fatal cancer types affect human internal organs, including bronchial tubes, lungs, ovaries, mammary and prostate glands.

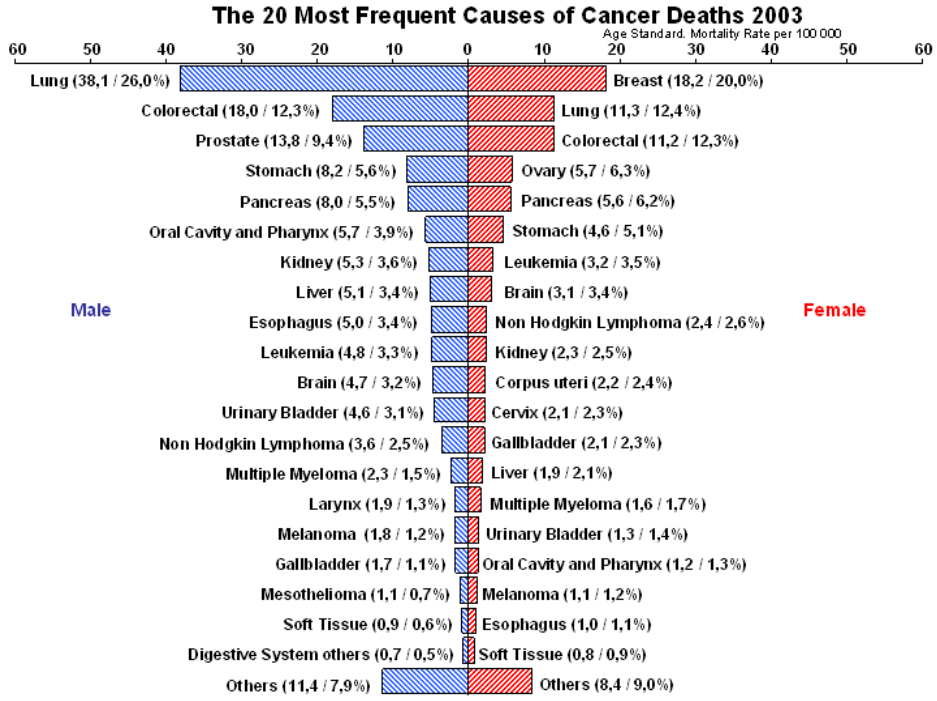


Figure 1 Cancer Mortality by Tissue - Leading Causes of Cancer Deaths. This figure represents age standardised cancer mortality rates in the Federal Republic of Germany (taken from http://www.dkfz-heidelberg.de (DKFZ 2003)). Lung cancer is the leading cause of death in males and breast cancer is the leading cause in females. The most common types of cancer are – with the exception of gender-specific cancer types - largely identical.

Cancer Therapy: Conventional Approaches – Conventional cancer therapy is based on a combination of surgery, and pre- or postoperative radiation- and chemotherapy. Chemotherapy shows significant success in the treatment of childhood leukaemia, but for epithelial tumour types that constitute about 80% of all tumour-related deaths, chemotherapy neither improves the patients' quality of life nor increases life expectancy (Abel 1996). Most cytostatic drugs applied in chemotherapy

aim to damage DNA in order to stop proliferation of cells characterised by high cell division rates. This is the reason why cytostatic drugs exhibit a broad range of side effects, including potential damage of liver and heart, as well as immune suppression (Windstosser 1994). However, the genesis of all cancer types is to some degree due to precancerous chronic immune deficiency. Therefore, the benefit of cytostatic drugs in cancer therapy is questionable (Schmähl 1986).

In contrast, the benefit of radiation therapy in pre- and postoperative treatment for the reduction of highly proliferating cancer tissue is indisputable. Nevertheless, destroying cancer tissue by radiation promotes the release of both cancer cell components and intact cancer cells into the blood stream. This increases the risk of metastasis formation and primarily, detoxification and waste disposal organs, such as kidney and liver, are affected by new tumour growth.

Recent Developments in Cancer Therapy – In order to overcome drawbacks arising from cytostatic drugs and radiation, modern cancer treatment optimises existing methods and introduces principles of adjuvant and neo-adjuvant therapy as a complement or alternative to established approaches. Adjuvant and neo-adjuvant therapy aims to reduce postoperative tumour regrowth or to transfer the tumour from a non-operable into an operable state (Jatzko 2005). Different methods, such as radiation-, chemo-, and immunotherapy, are applied alone or in combination. In particular, immunotherapy offers several promising principles. The most common approaches are listed in Table 1.

Principle	Description
Active immunisation	Immunisation against known tumour viruses
Non-specific active immunisation	Immunisation via synthetic molecules (cytokines: INFa,b,y, IL2, TNF)
Adaptive immunotherapy	Immunity transfer or tumour resistance transfer from one individual to another via in vitro cultivation of tumour-specific lymphocytes
Passive immunotherapy	Treatment with monoclonal antibodies against tumour antigens
Immunodepletive therapy	Transplantation of bone marrow

Table 1 Recent Approaches in Immunotherapy (Jatzko 2005).

In particular, theranostics, a recently emerging new field that combines **thera**peutics and diag**nostics**, introduces promising ideas to cancer therapy (Mazumdar-Shaw 2005). Initially, concepts of theranostics were applied to pharmaco-genomics. In 1995, a research group at Mayo Clinic, Rochester, USA described why the childhood leukaemia drug, Azathioprine, caused fatality in some patients, although the drug's efficiency was proven in most cases. It was reasoned that a missing enzyme, TPMT (Tiopurine methyltransferase), is responsible for the fatality observed in certain cases (Szumlanski 1995). This raises the question of whether or not it is sufficient to design drugs from a disease oriented perspective. A promising alternative might be to design specific drugs for individual patients or patient subgroups based on their genotype (individualised therapy).

Several research groups are addressing this question differently (Diaz-Rubio 2005; Hanrahan 2005; Shen 2005). However, many recent approaches are similar in that they utilize principles of theranostics and pharmaco-genetics. Here, they apply modern biomolecular techniques to investigate both, drug response and dose finding on a genomic and proteomic level. With the publications of the first draft sequence of the human genome in 2001 (Lander 2001; McPherson 2001; Venter 2001), and its subsequent completion, error correction, verification and annotation (Stein 2004), modern medical research possesses a powerful and vast knowledge base to support cancer research and therapy. In

order to deploy this information for the benefit of gaining deeper insight into the mechanisms and the aetiology of human diseases, it is essential to compare genomic information with other information about human diversity and cellular pathways. For that reason, efficient techniques need to be applied in order to extract genomic, proteomic or transcriptomic information, derived from messenger RNA (mRNA) splicing, translational and post-translational variations, epigenetics (histone code, DNA methylation etc.), protein sequence and structure, and protein-protein interactions. High-throughput technologies with the capability to rapidly characterise genomic functionality, constitute the basis of functional genomics (Bailey 2002). The information gathered via diverse techniques, including DNA sequencing, PCR, rtPCR and qPCR, DNA and protein microarrays, native chromatin immuno precipitation (NChIP), fluorescent in situ hybridisation (FISH), yeast two hybrid, mass spectrometry and many more (Ideker 2001), needs to be assembled. For that reason, powerful bioinformatics tools have been designed to help mine biological information, and make meaningful associations with clinical data. Hence, the combination of biological and clinical information collated via bioinformatics is critical for deriving near-term benefits in clinical research from the underlying basic biomolecular knowledge.

For instance, in 1997, the Food and Drug Administration (FDA) approved FISH technology for prenatal diagnosis (Tepperberg 2001). In breast, bladder and renal cancer, several microarray technologies were applied to rapidly identify associated genes, accelerate diagnosis and adjust therapy (Man 2004). However, the main objectives of theranostic approaches are to increase patients' life expectancy and quality of life, exclude ineffective or harmful medication by individually analysing the patient's drug and dosage response *in vitro* and therefore reducing hospital and medication expenses.

The Immune Efficiency Test (IET): A Theranostic Approach For Cancer Therapy – The IET was developed and first introduced by Dipl. -Ing. Hans-Albert Schöttler, specialist in general medicine, in 1999 and has been applied to more than 80 patients since then. The analytical part of the IET was performed in collaboration with Davids Biotechnologie GmbH in Regensburg. The test is an in vitro method analysing the ability of the patient's immune system to kill cancer cells under the influence of different drugs. Here the motivation is, to select the drugs which perform best in the in vitro test and design an individualised treatment. Most interestingly, the IET may also lead to accurate prediction of ideal medication, which is discussed in this paper.

The immune efficiency test combines personal diagnosis with individualised therapy and is therefore classified as theranostic approach.

Organising and Model Building of IET data – Medication prediction constitutes the central motivation of this IET-based study. Here, principles of theranostics and individualised therapy are combined with modern molecular biology and bioinformatics. This study covers the following four tasks.

(1) Documentation of the immune efficiency test.

(2) Design and implementation of a database to store and maintain IET data and supplemental patient information.

(3) Development and introduction of a web interface to provide local and remote access to the database.

(4) Knowledge extraction of IET data to test the hypothesis that predictive models estimating medication effects can be built.

The genesis of cancer is associated with immune suppression. Therefore, the fundamental idea of IET is that cancer therapy should improve immune activity. For this purpose, two properties are examined, namely the patients' general immunological constitution and

their individual ability to eliminate cancer cells. As a result, IET is a personalised analytical assay mimicking the interacting system of cancer and immune cells under varied medication on an *in vitro* platform.

Consequently, the purpose of this thesis is to provide a detailed documentation of the immune efficiency test, which has not been published yet. Furthermore, the infrastructure for organising and analysing IET data, which was designed and implemented within this thesis, is described. In addition, this document proposes two prediction models derived from two different machine learning algorithms, and describes all required dataset and attribute operations in detail.

2 Theoretical Fundamentals

2.1 Immunity and Cancer

All types of immune cells arise from bone marrow stem cells. Some develop into myeloid progenitor cells while others become lymphoid progenitor cells.

Myeloid progenitor cells develop into cells that respond early and non-specifically to infections. These include neutrophils, monocytes, macrophages, eosinophils and basophils.

Lymphoid progenitor cells develop into lymphocytes characterised by a later but more specific response to infections. After antigen-presenting by dendritic cells or macrophages, lymphocytes trigger a specifically tailored attack. Lymphocytes further differentiate into B cells, T cells, dendritic cells and natural killer cells.

When normal cells turn into cancer cells, some surface proteins (antigens) change. Cancer cells, like most cells, constantly release protein fragments into the circulatory system. Tumour antigens among these fragments prompt an immunological reaction by activating natural killer cells and T cells. These cells provide a constant and body-wide surveillance and eliminate cells that undergo malignant transformations. Tumours develop when this surveillance breaks down or is overwhelmed (NCI 2005). For this reason, lymphocyte stimulation might support this surveillance and become a promising therapeutic option in cancer therapy (Dunn 2005).

2.2 The Immune Efficiency Test (IET) – A Theranostic Approach

As noted in Chapter 1, theranostics (personalised medicine) is the convergence of *therapeutics* and *diagnostics*. This neologism defines a diagnostic tests that can identify which drugs are most suited for a patient. Theranostic tools can provide physicians with information that

enables them to individualise and optimise a patient's therapeutic regimen (www.oralcancerfoundation.org 2004).

Immunotherapy in cancer treatment substantially necessitates the implementation of theranostics. The ability of the immune system to mount a response to disease is highly patient-individual and depends on interactions between the various components of the immune system and the antigens. The complexity of the immune system is reflected in the presence of approximately 200 different blood group substances. Blood groups are classified according to immunological (antigenic) properties and are placed within 19 known blood group systems, such as Lewis, Lutheran and MNSs. The most commonly used blood group system is the ABO system and the association of blood group substances to cancer is well reported (D'Adamo 2000; Madjd 2005; Schneider 2005).

The immune efficiency test (IET) is a theranostic approach supporting individual immunotherapy for cancer patients. The test individually analyses the lymphocytes' ability to detect and eliminate cancer cells under the influence of varied therapeutic substances. This results in a schema identifying drugs reducing or increasing lymphocytic immune response and consequently affecting cancer growth. This schema can be applied for individualised cancer therapy.

2.3 IET Technology

The IET probes the effect of various drugs on the interaction between cancer cells and human lymphocytes. On the one hand, particular drugs may directly affect cancer cells either by introducing toxic or growth inhibitory substances. Conversely, medication may affect the human immune system and promote immune suppression or enhancement. The IET mimics this interactive system on an *in vitro* platform by incubating lymphocytes and cancer cells, along with the drugs in question (Figure 2).

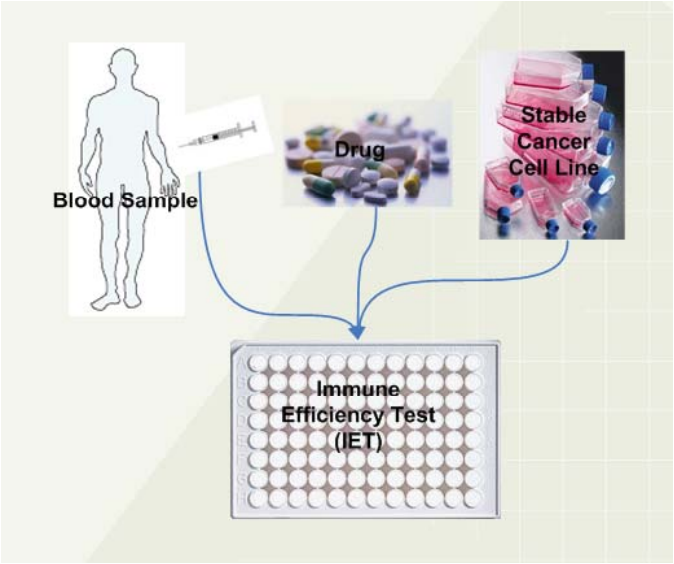


Figure 2 Schematic Illustration of the Immune Efficiency Test (IET). In each well of a 96 well micro plate, patient lymphocytes, a stable cancer cell line plus the respective drug in question is incubated and analysed.

The test is structured by four central steps as below:

(1) Lymphocyte Isolation - isolation of lymphocytes from patient blood samples

(2) IET *in vitro* Simulation – incubation of lymphocytes, cancer cells and drug

(3) Tetrazolium-based Reduction Assay – measuring cancer cell proliferation

(4) Interpretation and Therapy – interpretation of the results and optimisation of the therapeutic regimen

Lymphocyte Isolation – Purification of lymphocytes is based on an optimised Ficoll density separation (Bennet 1994; Bernhanu 2003). The isolation of peripheral mononuclear cells (PMNCs) is traceable to a method that Bøyum established in 1968 (Bøyum 1967-68).

Cellular blood components, including granulocytes, lymphocytes, monocytes, thrombocytes and erythrocytes can be separated according to their different physicochemical or biochemical properties. These include size, density, surface charge, adherence, antigen expression and phagocytosis activity (Wegener 1998). Lymphocyte isolation applies

density gradient centrifugation loosely based on the Meselson and Stahl protocol (Meselson 1958) originally developed for the investigation of DNA replication. This method separates cells of different size and density via centrifugation. Ficoll (specific weight: 1.077 g/ml) is a water soluble synthetic high molecular weight (M_w = 400000 g/mol) polymer based on sucrose and epichlorohydrin. While erythrocytes and granulocytes pass the gradient during centrifugation (800g, 15 min) due to their higher specific weight, lymphocytes, monocytes and thrombocytes stay above the gradient and the lymphocyte enriched section of the inter phase can manually be separated from the rest. The lymphocytes isolated can then be used for further investigations.

IET *in vitro* Simulation – Each single IET experiment simulates the interacting system of an immunological reaction. In this study, the immunological *in vivo* system is mimicked on a micro plate platform. Here the concept of a *micro array of cells* serves as a model for IET technology. The transfer from the micro plate to a micro array platform is intended for the near future.

In 2001, Ziauddin and Sabatini described a high-throughput tool for the analysis of gene over-expression called "transfected cell microarray" (Ziauddin 2001). Furthermore, cell microarray technology is applied to siRNA (small interfering RNA) analysis (Kumar 2003; Mousses 2003) and is integrated in several projects, including initiatives by the Mammalian Gene Collection (MGC) (Straussberg 1999) and the Harvard Institute of Proteomics. This aims to determine potential targets for human diseases. The principle of a high-throughput cell microarray, as Xu and colleges presented in 2002, serves as a model for the technology applied in this study (Xu 2002).

The IET determines the lymphocytes' ability to eliminate cancer cells under the influence of various therapeutic substances by monitoring alterations of the proliferation rate of cancer cells. In order to successfully mimic the *in vivo* system, lymphocyte concentration of the assay and of the blood sample need to be identical. The principle of

identical *in vivo* and *in vitro* conditions applies to the medication conditions as well.

Tetrazolium-based Reduction Assay – Measurement of cell proliferation and cell viability forms the general basis for many *in vitro* assays of a cell population's response to external factors. The proliferation rate of the cancer cell line used in IET analysis is determined via a tetrazolium-based reduction assay. This assay is based on the ability of metabolically active cells to reduce tetrazolium salts to formazan (Aziz 2005). After 3-4 hours of incubation, formazan crystallises in early apoptotic and living cells. By adding organic solvent, cell lysis is induced and the crystal structure of formazan dissolves. Formazan solution absorbs light at a specific wavelength. Cancer cell proliferation depends on lymphocyte activity and medication. For that reason, absorbance is a measure for cancer cell proliferation and provides a means to determine individual patient response to a particular drug.

Interpretation and Therapy – As previously explained, the IET simulates the immunological reaction of lymphocytes on an *in vitro* platform. This gives information about 1) the patient's individual immunological condition and 2) the effect of a particular drug on the lymphocytes' ability to eliminate cancer cells. The first is assessed by monitoring the patient-specific lymphocyte concentration in the blood sample. The latter is probed by performing both standard reactions without drug addition and test reactions with drug addition on the same micro plate. The proliferation values of test and standard reactions are normalised by the proliferation value of cancer cells without any lymphocyte and drug addition. Therefore, all proliferation values – measured as optical densities (ODs) – represent relative changes of the proliferation when cancer cells alone are compared to treated cells (see Equation 1).

$$ILA = \frac{\left(OD_{Drug} - OD_{Control}\right)}{OD_{Control}} - \frac{\left(OD_{STD} - OD_{Control}\right)}{OD_{Control}} = LA - SPR$$

ILA	:	*Induced Lymphocyte Activity*
LA	:	*Lymphocyte Activity (normalised)*
SPR	:	*Standard Proliferation Rate (normalised)*
OD_{Drug}	:	Optical Density of the *Test Reactions*
$OD_{Control}$	:	*Optical Density of the Control Reaction*
OD_{STD}	:	*Optical Density of the Standard Reaction*

Equation 1 Calculation of Induced Lymphocyte Activities (ILA). ILA values are derived by 1) comparing and then normalising optical densities (ODs) of test and standard reactions by the OD of the control reaction and 2) subtracting the normalised standard values (SPR) from the normalised test values (LA).

The proliferation rates of the standard reactions represent the patients' own ability to eliminate cancer cells without the aid of drug addition. These values are henceforth called **S**tandard **P**roliferation **R**ates (**SPR**, see Equation 1 and Figure 3). The effect of a particular drug on the lymphocytes' ability to eliminate cancer cells is assessed by determining the proliferation rate of cancer cells under the influence of varied drugs when lymphocytes are present. The normalised values of these reactions are called **L**ymphocyte **A**ctivity (**LA**). LA subtracted by the SPR, represents the pure contribution of the drug to cancer cell elimination/stimulation (some drugs may also stimulate cancer proliferation). These values are called **I**nduced **L**ymphocyte **A**ctivity (**ILA**). The terminology of *lymphocyte activity* and respectively *induced lymphocyte activity* refers to the fact that 1) the ability of lymphocytes to eliminate cancer cells can also be interpreted as lymphocyte *activity* and 2) medication can *induce* an increase or decrease in lymphocyte activity.

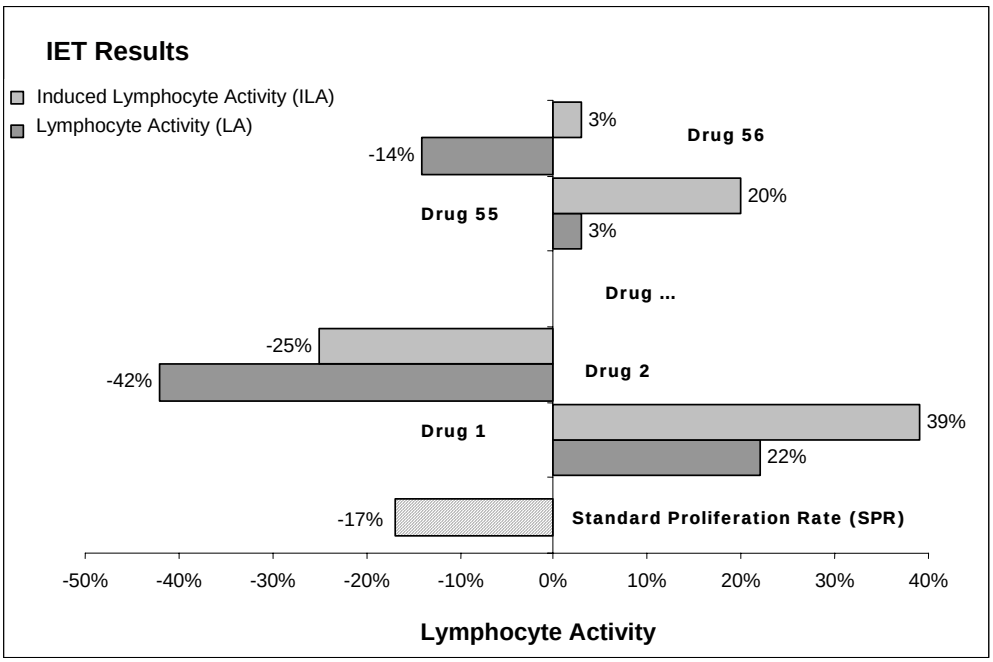


Figure 3 Interpretation of IET Results. To determine the pure drug effect, the Standard Proliferation Rate (SPR) is subtracted from the *lymphocyte activity* (LA) of the drugs 1-56. This results in *induced lymphocyte activity* (ILA) values, which are previously normalised by the proliferation rate of cancer cells without lymphocyte and drug addition.

In order to optimise and adjust a patient's therapeutic regimen, the ILA values of the various drugs are crucial. Drugs being considered for treatment need to show negative ILA values. This means that only drugs that increase the reduction of cancer cell proliferation when compared to the patient's own ability (SPR) to eliminate cancer cells are chosen for treatment. In Figure 3, only *Drug 2* fulfils this requirement. All other drugs show positive ILA values. For instance *Drug 1*, *Drug 55* and *Drug 56* even enhance cancer cell proliferation, although *Drug 56* seems to decrease cancer cell proliferation (LA). But when compared to the SPR, it becomes apparent that *Drug 56* induces cancer cell proliferation.

2.4 Database Technology

Motivation – The history of database systems goes back to libraries, governmental, business and medical records. There is a long tradition of information storage, indexing and retrieving. Basically two different approaches of information management systems are distinguished, namely file-based systems and database systems (Connolly 2001). A file-based system constitutes a collection of application programs performing services for the end user. Each of these programs defines and manages its own data. Limitations of this file-based approach are summarised in Figure 4.

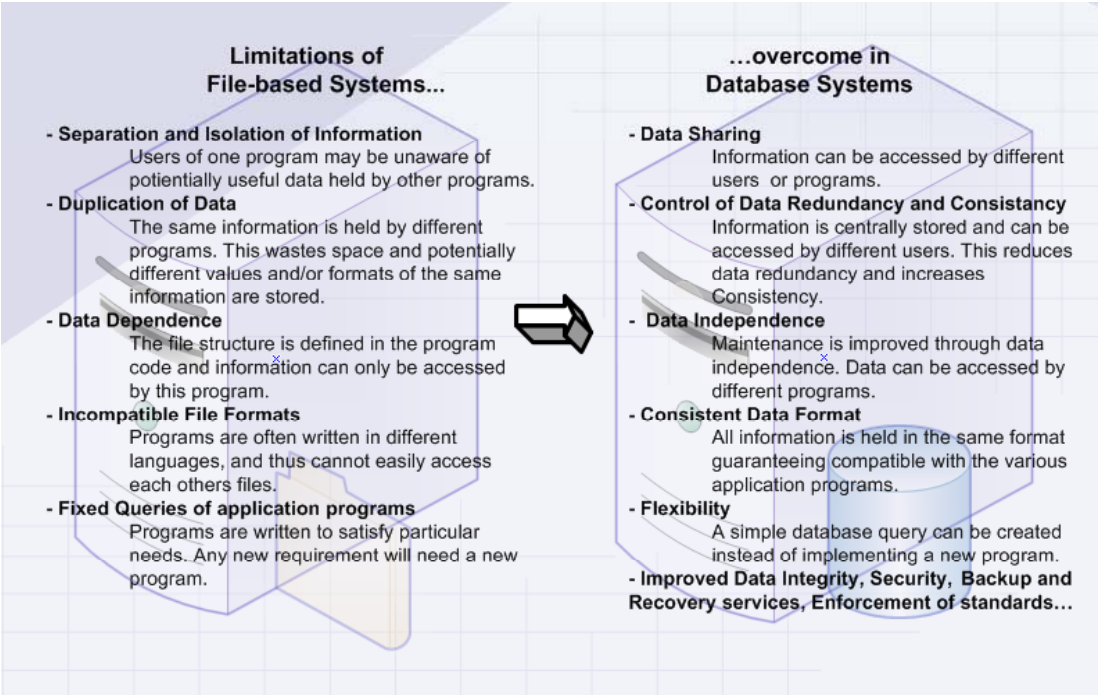


Figure 4 Turning Limitations into Innovations. Database systems turned limitations of file-based systems into innovations and are now the most commonly used data repository systems.

To overcome and prevent these limitations of file-based systems, the database approach arose (Figure 4). Here, data is no longer simply embedded in application programs but stored separately and independently. This implies access and manipulation of data beyond that imposed by application programs. A database is a shared collection of logically related data, designed to meet the information needs of an organisation. Here, a system catalogue provides metadata to enable

independence of program data. A software system that enables users to create, define and maintain databases, and which provides controlled access to this database, is called a *database management system* (DBMS). Three types of DBMSs are as follows: hierarchical and network-based DBMSs (first generation), relational DMBSs (second generation) and object-relational or object-oriented DBMSs (third generation). There are hundreds of DBMSs on the market such as MS Acess, MS SQL server, MySQL, Oracle, Postgre SQL and many more.

Drawbacks of DBMSs are mainly due to a higher degree of complexity, which is accomplished by the demands of trained users. Nevertheless, the advantages of DBMSs significantly exceed the drawbacks as illustrated in Figure 4 and are therefore used for information storage in this study.

Relational Database Model – The relational model was developed from the work done by E. F. Codd in the late 1960s (Sol 1998) and now constitutes the most commonly used database model. The fundamental structure of the relational model is the concept of tables (also called relations) in which all data is stored. Each table constitutes of records (horizontal rows, also called tuples) and fields (vertical columns, also called attributes) and is identified by a unique key attribute (primary key). This key is essential for the DBMS to organise and find records. This distinguishes the relational model significantly from hierarchical or network-based models, in which the user is responsible for data structure within the database. Querying of relational databases is simply done by comparing the value stored within a particular column and row to some criteria. Users can query information concerning table names, access rights, storage types etc. This simplifies administration of the database, as well as usage.

Database Design Methodology – The database design methodology is a structured approach using procedures, techniques, tools and documentation aids to support and facilitate the process of design. Database design is structured as follows:

(1) **Conceptual Database Design –** Conceptual database design is the process of constructing a model of information used in an enterprise, independent of all physical considerations. Here, the "real-world" domain is specified and captured. All entity and relationship types are identified and associated with the respective attribute types. This is done by using a database design model (Entity-Relationship-Model – E/R model (Chen 1976)).

(2) **Logical Database Design –** Transformation of a conceptual model into local and global logical data models supported by the DBMS (Relational Model (Sol 1998)). Normalisation procedures ensure that information will be non-redundant and properly connected.

(3) **Physical Database Design –** The relational model is translated into tasks physically concerning the DBMS. Here, the implementation of the database is described including file organisation, indexing, user views, security mechanisms and analysis of disk space requirements, among others.

Separation of database design into these three phases reduces the complexity of database projects. If not all design decisions depend mutually on one another, problems can be separated and solved one after the other. In order to increase the efficiency of a database, normalisation is performed (logical database design). This is the process of increasing efficiency of a database by eliminating redundant information and assessing significance of data dependencies by a set of

rules. Most commonly four different normal forms are distinguished and defined as follows (Kennedy 2000).

- **Un-normalised Form (UNF) –** A table that contains one or more repeating groups.
- **First Normal Form (1NF) –** A relation in which intersection of each row and column contains one and only one value.
- **Second Normal Form (2NF) –** A relation that is in 1NF and every non-primary-key attribute is fully functionally dependent on any candidate key.
- **Third Normal Form (3NF) –** A relation that is in 1NF and 2NF and in which no non-primary-key attribute is transitively dependent on any candidate key.
- **Boyce-Codd Normal Form (BCNF) –** A relation that is in 3NF and all determinants are candidate keys.

In practice, the boundaries of the three design phases are overlapping and even de-normalisation is a common means to introduce a different logical model for performance purposes (Angus 2001). In addition, adjustments or alterations of the physical design (software and/or hardware) may be required to meet performance requirements. This can easily be done, if database design was conducted in separate phases. In general, separation of database design into these three phases increases flexibility and simplifies implementation of large database projects.

2.5 Web Interface

For the interaction of a client with a data repository system, several approaches including *mainframe* and *two tier architecture* are available. However, the most commonly used client-server architecture is *three tier client-server architecture* (also known as multi tier architecture, Figure 5). The first tier (client) contains the presentation logic, including simple control and user input validation. The second tier (application server), provides the business logic and data access. Tier three (database server), provides the business data.

This architecture enables easy modification and replacement of one tier without affecting the others. Additional, separating application and database functionalities enhances load balancing and security policies can be enforced within the server tiers without interfering with the client (Ramirez 2000).

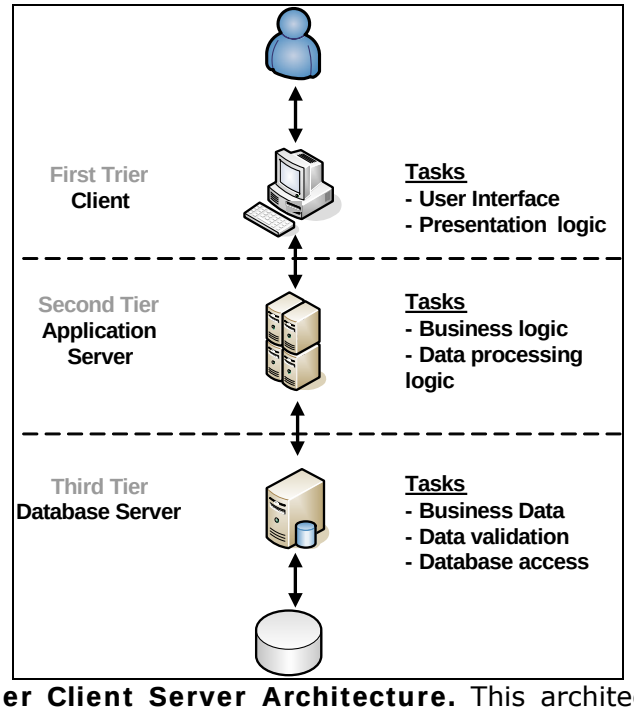


Figure 5 Three-Tier Client Server Architecture. This architecture separates the three domains *Client*, *Application Server* and *Database Server*.

Most web applications accessing a database directly via a browser (web interface) use three tier client-server architecture. For communication of DBMSs with the Web, several approaches are available. Along with

Common Gateway Interface (CGI) technology, the use of scripting languages (JavaScript, VBScript, Perl and PHP) extending browser and web server with database functionality are the most common techniques.

The world wide web (www) constitutes the most popular and powerful networked information system to date. The www was designed to be platform-independent and therefore, web-based applications can significantly lower deployment and implementation costs. Web sites today contain more and more dynamic information. The content of a dynamic Web page is generated each time it is accessed. This enables the web page to respond to user input and to provide customised views. However, accessing a database via the web (Web-DBMS Approach) involves some drawbacks, primarily those concerning reliability and security. Nevertheless, the Web-DBMS approach is a simple and platform-independent application to access a database via the Web.

2.6 Data Mining and Knowledge Discovery in Databases

"Data Mining is the process of exploration and analysis, by autonomic or semi-autonomic means, of large quantities of data in order to discover meaningful patterns and rules." (Johansson 2004)

Several terms have been put forth to describe the process of discovering useful patterns in data. These include data archaeology, data mining, data pattern processing, information discovery and information harvesting (Fayyad 1996). The term *data mining* has in particular been used by statisticians and data analysts, but it is now also commonly used in the database field. The term *knowledge discovery in databases* (KDD) emphasises the fact that knowledge is the result of a data-driven discovery (Piatetsky-Shapiro 1991). In general, *KDD* is the overall process of discovering meaningful knowledge. One step of this process is *data mining* which aims to discover and extract underlying patterns from data through the application of specific algorithms (Brodley 1999). The central motivation of the KDD approach

is to transform low-level data into a more abstract, compact and useful format. This may result in a model of the process that generated the data, a brief report or a prediction model to estimate future values. *KDD* is an iterative process comprising the following six stages.

(1) Basic Understanding of the Enterprise

(2) Dataset Creation

(3) Correction or Removal of Corrupted Data

(4) Data Reduction

(5) Model Building by the Use of Data Mining Algorithms

(6) Interpretation and Validation of the Results

These operations are summarised in CRISP-DM 1.0 (Chapman 1999) a widely accepted methodology to design and describe the KDD process.

2.6.1 Data Mining Algorithms

Data mining is an AI-powered procedure that aims to discover meaningful information from a data source that then can be used to improve action. To achieve this objective data mining uses machine learning algorithms. Machine learning refers to a process or system capable of autonomously acquiring and integrating knowledge (AAAI 2006). Both machine learning and data mining are subtopics of AI. In 2004, John McCarthy defined intelligence as the "*…computational part of the ability to achieve goals in the world. Varying kinds and degrees of intelligence occur in people, many animals and some machines…*" (McCarthy 2004). This is a general – even so anthropological – definition of intelligence and enables us to explain the term AI. The American Association for Artificial Intelligence describes AI as "*…the scientific understanding of the mechanisms underlying thought and intelligent behaviour and their embodiment in machines…*" (Reddy 1996). The technical implementation of AI results in the development of algorithms, such as flexible rule interpreters, artificial neural networks (ANNs) and self organising software (Sloman 1998).

AI-powered algorithms are implemented in several data mining software tools which most commonly provide unsupervised (grouping into similar, initially undetermined classes) and/or supervised (predictions using already determined classes) machine learning algorithms (Mangasarian 1990; Mangasarian 1990; Wolberg 1990; Bennet 1992). Both types – supervised and unsupervised – are implemented in Clementine (SPPS) providing implementations of *Build C5.0*, *classification and regression tree* (CART), *ANN* and *Kohonen* algorithms. However, many other comparable software tools are available, such as Darwin (Thinking machines, Corp.), Intelligent Miner (IBM), CART (Salford Systems) (Elder 1998)

Several prediction methods have been published using machine learning in gene expression profiling (Golub 1999; Brown 2000; Furey 2000). These studies demonstrate the substantial demand of restricting prediction models by taking account of the competing demands of simplicity and accuracy (Brazam 2000). This principle applies to immune efficiency profiling as well. Therefore, this study focuses on the application of the two supervised machine learning algorithms *ANN* and *CART* as described by Dudoit *(Dudoit 2002).* The algorithms are discussed below.

Artificial Neural Networks (ANNs) – *ANN* algorithms simulate neuronal information processing of the human brain at a very basal level. *ANNs* – as their human paradigm – are able to learn and generate expert abilities on a trial and error basis. Table 2 summarises and Figure 6 illustrates analogies between artificial and biological neural networks.

Biological Neural Network	Artificial Neural Network
Soma	Neuron
Dendrite	Input
Axon	Output
Synapse	Weight

Table 2 Analogy between Biological and Artificial Neural Networks.

Learning of *ANNs* is performed with a large number of single subunits (neurons) organised in different layers (usually input-, middle- and output-layer). The linkage of these subunits is implemented by variable

connection weights (Figure 6). Based on the investigation of several individual data points, an *ANN* formulates predictions for each single record and adjusts the previously defined connection weight in case the prediction was incorrect. The definition of stopping criteria enables the user to terminate this self-repeating process (SPSS 1994-2003).

Neurons constitute the central processing units of an ANN and are organised as illustrated in Figure 6. A neuron receives several weighted input signals, computes a new activation function and sends the result to its output link. For instance, the output is "–1" if the weighted sum of the input signals is less than a certain threshold. If the input is higher than the threshold, the neuron becomes activated and the output attains "+1". This particular activation function is called *sign function*. However, several other activation schemes have been tested, including step, linear and sigmoid functions (Negnevitsky 2002). Depending on the activation function chosen and the resulting output signal, the input weights are updated differently. This process is performed iteratively.

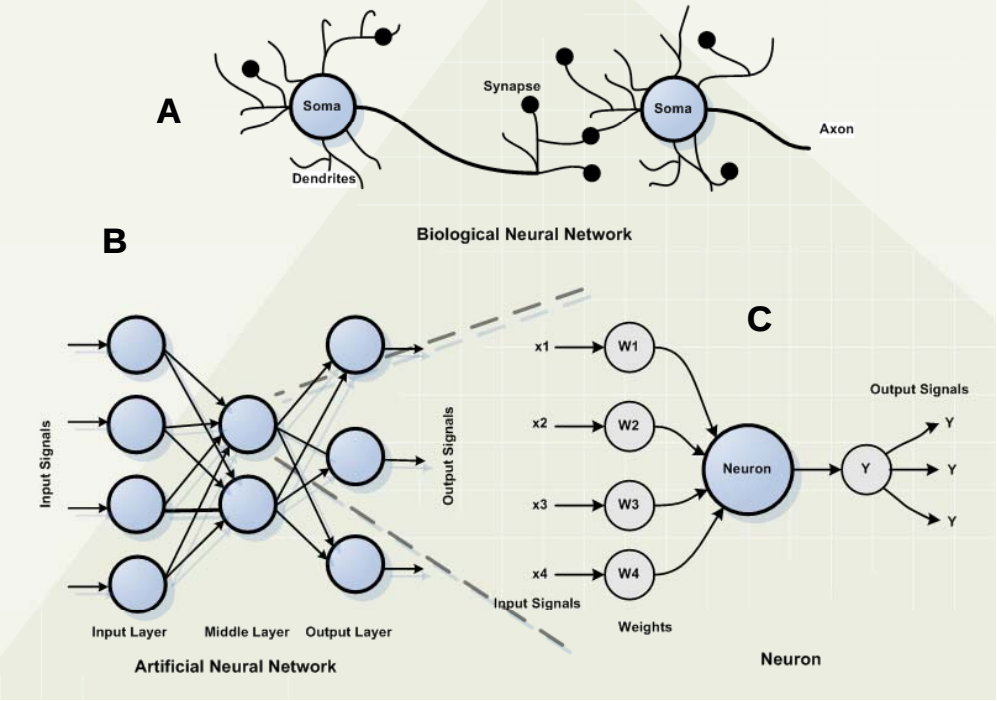


Figure 6 Architecture of Biological and Artificial Neural Networks. Section A) depicts a biological neural network, B) the basic architecture of an artificial neural network and C) illustrates a single neuron of an artificial neural network.

In conclusion, the training process of ANNs is performed in four steps:

(1) Initialisation – The initial weights (w_1, w_2,…,w_n), a certain threshold and the activation function are defined.

(2) Activation – The neuron output is calculated based on the activation function chosen and the respective weighted input.

(3) Weight training – The input weights are updated according to the difference between the respective output and the expected value.

(4) Iteration – Iteration starts at step 2 and is repeated until convergence.

Classification and Regression Tree (CART) – CART is a decision tree algorithm and "*…takes as input an object or situation described by a set of properties, and outputs a yes/no decision…*" (Russell 1995). Decision making of CART algorithms is therefore based on Boolean functions. CART is a predictive rule induction algorithm that estimates future values by using binary recursive partitioning. The original records are split into fragments of similar output values. These partitions are ranked in order to find the best fragment. This process proceeds until a stopping criteria is reached. Most commonly, CART uses the *Gini coefficient*[1] as criterion to drive splitting (Luan 2002; Ranawana 2006), but also the tree-depth is a means of defining the termination of recursion. In general, rule induction algorithms cull through a set of predictors by successively splitting the data set of choice into subsets based on interactions of predictor and output fields. Recursive splitting results in the construction of decision trees (Sessum 2001).

Figure 7 illustrates a four layer decision tree generated by using a CART algorithm and built upon data derived from the breast cancer database established at the University of Wisconsin Hospitals (Madison, USA) by

[1] The Gini coefficient is a dispersion measure to analyse any form of uneven distribution. This statistical measure was developed by the Italian statistician Corrado Gini and his paper "Variabilità e mutabilità" was published in 1912 (http://en.wikipedia.org/wiki/Gini_index).

Dr. William H. Wolberg (Mangasarian 1990; Mangasarian 1990; Wolberg 1990; Bennet 1992). Based on cellular properties, the tree predicts a binary class label holding the values "2" (benign) and "4" (malignant) with an accuracy of ~96%. The tree shows that the *Uniformity of Cell Shape* attribute is most significant for predicting the class label. For 94% of the patients being considered "benign", the *Uniformity of Cell Shape* attribute is characterised by a value lower than 3.5. Conversely, 87% of the patients being considered "malignant" show values of higher than 3.5. This reflects the binary nature of the CART algorithms.

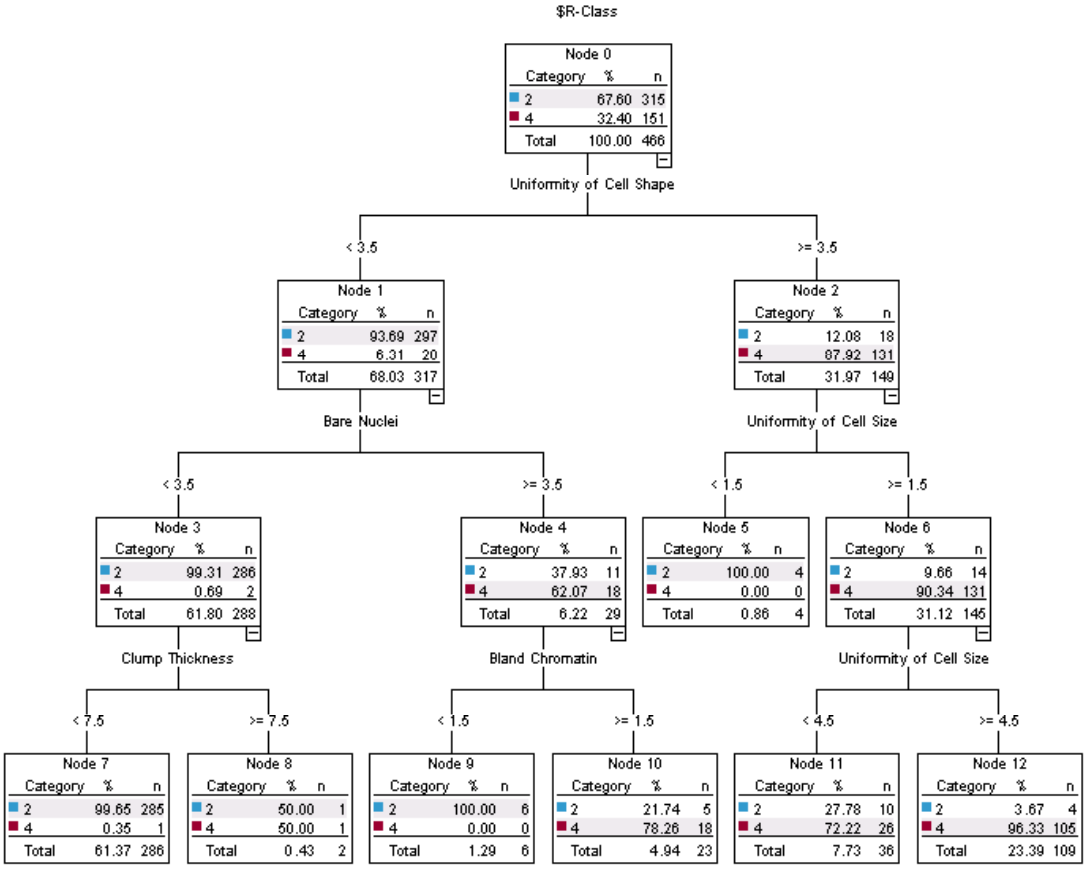


Figure 7 Decision Tree Built by Applying a CART Algorithm. The data are derived from the breast cancer database established at the University of Wisconsin, Madison. Each instance has one of two possible classes: "2" for benign and "4" for malignant. All other attributes are discrete numeric attributes with a range from "1" to "10", where "1" indicates that the attribute can not be applied to the respective instance and "10" that it can be fully applied.

Conclusion. While CART algorithms are easy to train, represented visually by trees and therefore provide interpretable models of the underlying data sets, ANNs are usually characterised by a faster response and lower computational times, but lack explanation facilities

and act as a black box. They essentially are models without information concerning the internal components or algorithms of the storage device (Wang 2004).

2.6.2 Limitations of Data Mining

While data mining is a significant advance of the analytical tools currently available to analyse large data sets, they are not self-sufficient applications and there are limitations to their capability (Seifert 2004).

Although data mining can aid in revealing patterns and relationships, it does not assess the significance of these patterns in a causal sense. This type of determinations can not be automated in data mining and must be performed by analytical personnel. The validity of the patterns depends on how well "real world" situations are explained. Data mining can identify connections and different connection weights between variables and the patterns discovered might describe the real world situation accurately but it does not necessarily increase the knowledge about the nature of the problem and how it is caused (Seifert 2004).

For successful data mining, skilled analytical personnel is required to structure the analysis, interpret the output and formulate meaningful results. Consequently, personnel or data limitations do primarily affect the success of data mining, rather than technical constraints.

In this chapter, four different techniques are described to overcome some of the aforementioned limitations.

(1) **CRoss Industry Standard Process for Data Mining (CRISP-DM) –** to structure data mining projects.

(2) **Hypothesis Testing for Statistical Dependencies –** to detect potential patterns before modelling algorithms are applied.

(3) **Validation by a Training and Validation Set Splits –** to investigate how patterns discovered compare to unknown circumstances.

(4) **Randomisation Testing –** to assess significance of patterns discovered.

CRISP-DM – CRISP-DM methodology was published by NCR Systems Engineering Copenhagen, DaimlerChrysler AG, SPSS Inc. and OHRA Verzekeringen en Bank Groep B.V in 1999 (Chapman 1999). It combines an industry proven guideline for data mining projects with helpful advices for proper documentation of the process and for clean presentation of the results.

In CRISP-DM methodology, the terminology slightly differs from the initially mentioned KDD process. In CRISP-DM, the term *data mining* refers to the entire process of knowledge discovery and the actual *data mining* phase, as defined above, is called *modelling or model building.* Nevertheless, CRISP-DM methodology constitutes the most commonly used standard protocol for knowledge discovery.

CRISP-DM methodology is best displayed as a life cycle model (shown in Figure 8). The model includes six phases, *business understanding*, *data understanding*, *data preparation*, *modelling*, *evaluation* and *deployment*, with arrows indicating the most important dependencies. The sequence of the phases is not strict and allows the analyst to go back and forth as needed. This guarantees flexibility for the users' diverse needs.

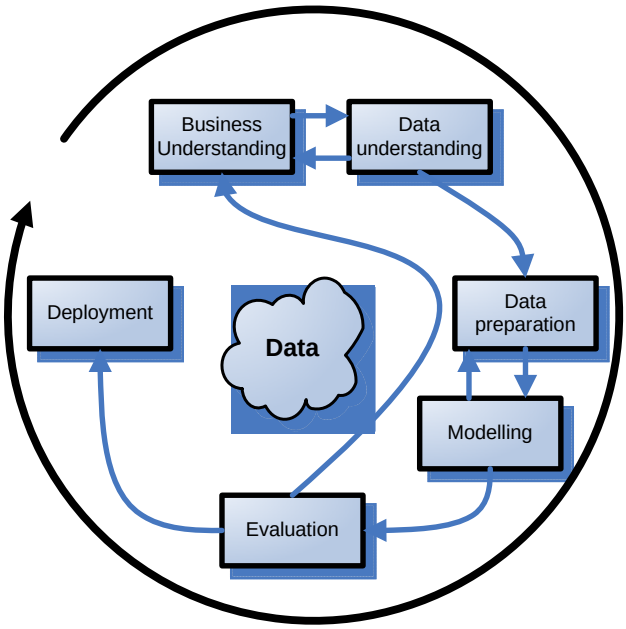


Figure 8 Phases of the CRISP-DM Reference Model. CRISP-DM includes the following six phases: business understanding, data understanding, data pre-processing, modelling, evaluation and deployment. (Chapman 1999)

The surrounding outer circle in Figure 8 refers to the cyclical nature of a data mining project and emphasises that a data mining lifecycle does not necessarily terminate, once one turn is finished. In most cases, deployment solutions trigger new questions for the previous phases such as *business-* or *data understanding*.

The initial phase of *business understanding* guides the analyst of a data mining project from a basic understanding of the diverse requirements and objectives to a first problem definition and a design of preliminary concepts for solving the problem from a business-oriented perspective.

Data understanding entails the intensive investigation of the initial dataset in order to assess data quality and to become aware of the type and format of information. Moreover, this process provides a first insight into the dataset and promotes the discovery of interesting subsets, first hypotheses and/or constraints. Without the use of machine learning algorithms, *data understanding* explores the dataset on a basal level and feeds into *data pre-processing*. Main tasks concern dataset, data and attribute considerations.

In-depth *data understanding* enables the analyst to proceed with *data pre-processing*, which covers activities such as assortment of significant records, attributes and subsets while disposing irrelevant information. Furthermore, in many cases data transformation and formatting is required to construct a dataset compatible with the modelling algorithms of choice. In cases of high-dimensional feature space, data pre-processing needs to be performed in two steps: First, pure data preparation, including data formatting, integrating, selecting, cleaning and constructing tasks; Second, a feature selection phase, to reduce feature space dimensionality (Piatetsky-Shapiro 2003). Here, several approaches such as *T-test for Mean Difference*, *Stepwise forward selection* or *stepwise backward elimination* are available (Han 2001).

The *modelling phase* applies various data mining algorithms to the dataset and adjusts respective parameters such as pruning or stop criteria to build significant models. Typically, different algorithms require different data formats and consequently stepping back to the *data pre-processing* phase may be required.

After successful model building, it is important to evaluate the model before *deployment*. The evaluation phase reviews all actions of the previous phases and probes whether or not the model takes all initially defined business objectives into account. Most importantly, quality and significance of the models generated are assessed here.

Deployment is the final phase of a data mining project which makes use of the findings, models and documentations of the study. In most cases, the deployment phase is not carried out by the analyst, but by the customer.

Hypothesis Testing for Statistical Dependencies. Assuming underlying patterns to the data given – and only then will model building be successful – statistical dependencies within the data will be detectable as well. By introducing hypothesis testing before model building is performed, the success of data mining algorithms on a given dataset can be investigated. On the one hand, this increases data mining

efficiency, since irrelevant datasets or subsets can be excluded and on the other hand patterns discovered during model building are confirmed. To prove statistical dependencies, the principle of linkage or gametic disequilibrium can be used. Linkage disequilibrium was defined by Brown and colleges who employed this concept to detect associations of *Hordeum Spontaneum* alleles among different loci (Brown 1980). This principle is used in LIAN 3.0 (from LInkage ANalysis), a software tool to test the null hypothesis of linkage equilibrium for multilocus data (Haubold 2000). LIAN was originally used to detect linkage disequilibrium in bacterial populations (Haubold 1998), but can be applied to any multilocus data such as IET data. Statistically relevant associations or linkage disequilibrium can be assumed if the null hypothesis can be rejected.

Validation by Training/Validation Set Splitting. Splitting the initial dataset into training and validation sets enables the analyst to perform model building and validation on two separate datasets. In doing so, it can be investigated how patterns discovered during model building compare to unknown circumstances. This mimics a "real world" situation. Most commonly, training and validation is performed with a 70%/30% randomly split dataset (Gansky 2003; Piatetsky-Shapiro 2003; Bloom 2004; Chakraborty 2005).

Similarly, in cases of high feature space and low number of instances, cross-validation can be performed (Radmacher 2002).

Randomisation Testing. Validating by training/validation set splits, however, is not sufficient for assessing the significance of a model. A small error rate found during validation does not necessarily guarantee that the patterns are significant (Radmacher 2002). To assess significance, it needs to be tested whether or not the models built perform significantly better than due to chance alone. This can be done using randomisation testing (Manly 1991).

For instance, in this study, 1241 immune efficiency profiles were used for model building. Five different class labels can be assigned to each

profile. This results in $5^{1241} \approx 10^{867}$ possible combinations for predicting the outcomes of the 1241 profiles. This number is too huge for any computer to analyse all possible permutations. Thus, a large sample (e.g. $N=10^5$) is used to estimate the proportion of random predictions that have an accuracy better than the model. This is done by randomly shuffling the actual class labels (ACLs) N-times and thus generating N randomised class label (RCL) sets (Figure 9). The percentage of RCL sets characterised by a higher accuracy than the predicted class label (PCL) set generated by the model is represented by the p-value. For instance, a p-value of 0.25 indicates that 25% of the RCL sets performed just as well as or better than the model. Therefore, the model may not be considered as significantly describing the underlying dataset.

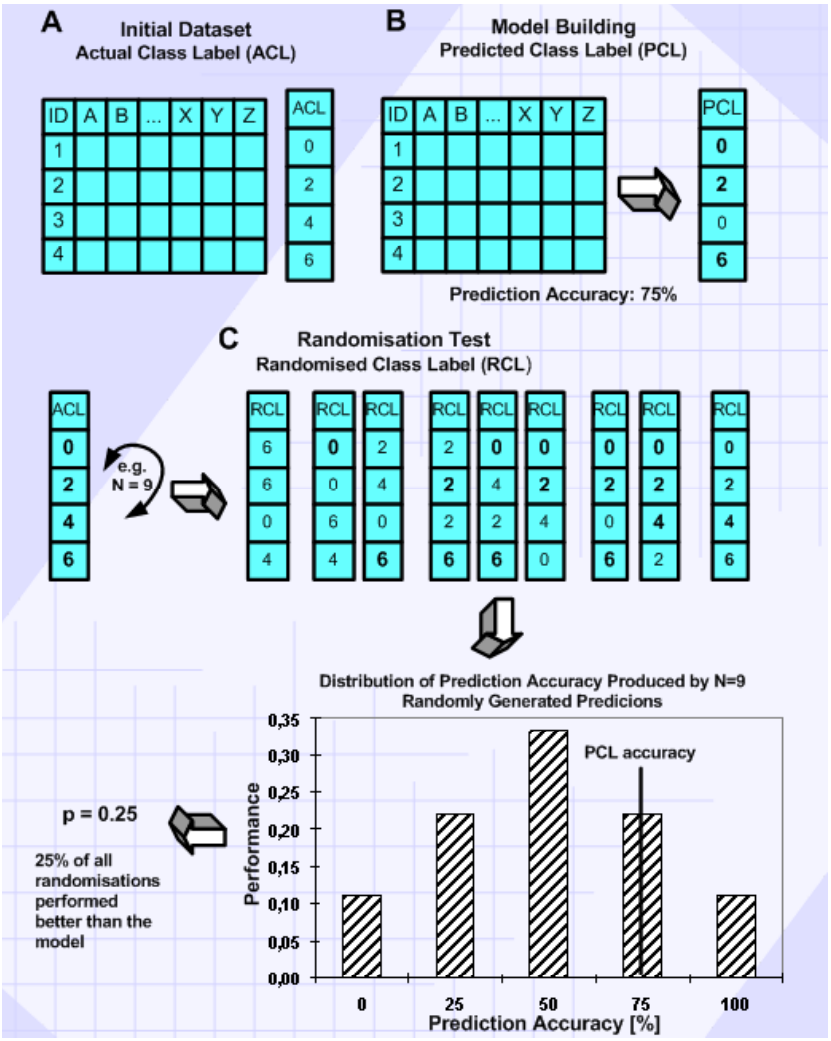


Figure 9 Randomisation Testing. (A) depicts the initial dataset and shows the actual class labels (ACL) for four instances. Model Building (B) generates predicted class labels (PCL) based on the supplemental information (attributes A to Z). Randomisation testing (C) shuffles the ACLs N times and consequently generates N randomised class labels (RCL). Comparison of RCL to ACL results in the distribution of prediction accuracy. The p-value represents the portion of the distribution that exceeds the accuracy level achieved by model building (or the p-value is the percentage of RCL sets performing better than the PCL set).

3 Materials and Methods

This section summarises all materials and methods required for this study. This includes technology to 1) perform the IET test; 2) create a relational database to store IET results along with clinical and general patient information; 3) implement a web-interface to access the database and 4) build predictive models to estimate drug effects.

3.1 Immune Efficiency Test (IET)

The IET analyses the proliferation rate of a stable cancer cell line under the influence of various drugs and patient individual lymphocytes. The proliferation rate is a measure to determine the usefulness of particular drugs for a specific patient. The test is performed in the following three steps:

Lymphocyte Isolation - Based on optimised Ficoll density separation, lymphocytes were isolated from whole blood using Ficoll-Paque™ Plus and following the manufacturers' instructions (Amersham Bioscience, Uppsala, Sweden).

IET Incubation – Cancer cells of a stable and well defined cancer cell line (details on the cancer cell line can not be published) were incubated at 37°C in a 96 well micro array along with the drug under review and patient lymphocytes. The cancer cell concentration was adjusted to 10^6 cells per millilitre. The total volume was 200µl. Lymphocyte concentration corresponded to the concentration in the blood sample. Drug concentration reflected the physiological dosage of the drug. Culture medium was **D**ulbecco's **M**odified **E**agle **M**edium (DMEM) with additives.

Measurement of Cancer Cell Proliferation – Cancer cell proliferation was measured using Mosmann's MTT reduction assay (Mosmann 1983). For each sample three wells of a 96-well microplate were used. 100µl of the sample plus 10µl of MTT stock solution (5mg MTT/ml of PBS) was

incubated for three hours at 37°C. The absorbance was measured subsequently using a spectrometer at the wavelength specific for formazan absorbance.

3.2 IET – Database (IETDB)

IETDB was organised with a relational model and stored in MYSQL 4.0.23 relational database management system. The database was implemented via phpMyAdmin (phpMyAdmin 2.6.0-rc3), a commonly used platform for database implementation in MySQL. For database design Microsoft Office Visio Professional 2003 was used. The SQL code is presented on the IET CD.

3.3 IET – Web Interface

Three tier architecture was used for assessing IETDB via a web interface. The web interface connects to the IETDB and was constructed by HTML scripts running on an Apache/2.0.48 web server. Client interference with the database was implemented via php-embedded SQL statements. All logical operations were executed in php and by JavaScript code embedded in HTML code. The source code of the web interface can be found on the IET CD.

3.4 Data Mining Methods

Initial Dataset – All information available from patient charts (see Figure 10) was integrated into one dataset (Microsoft Excel format).

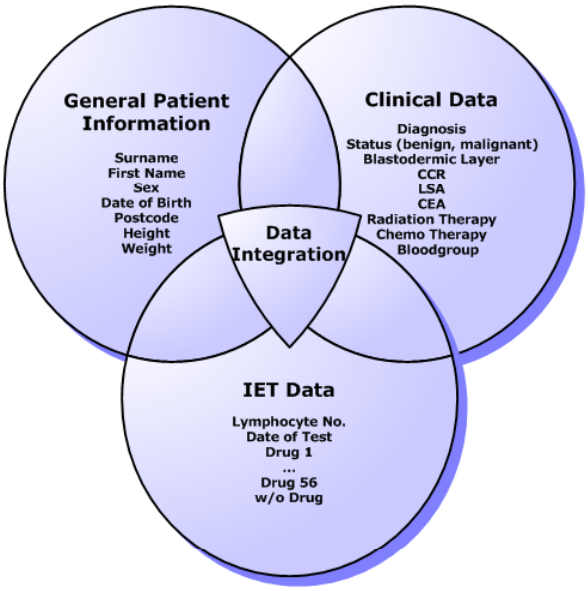


Figure 10 Structure and Attributes of a Patient Chart – The Basis for the Initial Dataset. The various attributes are sorted by the type of information they belong to. The three sources of information were integrated into one Excel file.

Consequently, this dataset was organised in a patient-wise manner (i.e. each single entry hold the complete IET result – for drug1 through drug56 – for a particular patient). Altogether, the dataset hold 105 entries for 75 attributes. Most attributes are self-explanatory, e.g. the attribute *Blastodermic Layer* can take "meso" for meso dermal, "ekto" for ekto dermal and "endo" for endo dermal. Data and storage types of all attributes are summarised in Table 3.

Attribute	Storage Type	Data Type
Status	String	Set
Patient ID	Integer	Set
Test ID	Integer	Set
Sex	String	Flag
Date of Birth	Date	Range
Date of Test	Date	Range
Postcode	String	Set
Diagnosis ID	Integer	Set
Blastodermic Layer	String	Set
Chemo Therapy	String	Set
Radiation Therapy	String	Set
CCR	Real	Range
LSA	Real	Range
CEA	Real	Range
Height	Real	Range
Weight	Real	Range
Blood Group	String	Set
Rhesus	String	Set
Lymphocyte No	Real	Range
Drug 1…56	Real	Range
w/o Drug	Real	Range

Table 3 Data and Storage Type Settings for the Initial Dataset.

For detailed information on the dataset and its attributes, a documentation file (IET_Documentation.info) was developed, which is now accessible via the IET database and can be found in Chapter 7 *Appendix*.

Data Mining Tools – To undertake data mining analysis of the data given, Clementine Graduate Pack 8.1 was used. In particular, performance of data pre-processing, modelling and evaluation was conducted in Clementine. For certain tasks of data pre-processing (Randomiser.jave and Transformer.java) and for model evaluation (RandomisationTester.java), java applications were developed using the java development kit jdk1.5.0_05. Basic functionalities of these tools are described in the results section and further details are presented in the appendix. The entire process of data mining was loosely based on CRISP-DM 1.0 methodology (Chapman 1999).

4 Results

The aim of this study was to document the immune efficiency test and to organise and explore IET data. At this point, more than 80 patients have been tested, their specific immunological reaction was monitored and based on these results an individualised therapy was applied.

Chapter 4 presents tools developed for organising and exploring IET data. For this purpose, a relational database was created incorporating IET, clinical and general data. To provide access to this database, a web interface was created. Finally, based on IET and supplemental information, knowledge discovery and data mining techniques were applied for model building. The motivation was to clarify whether or not models predicting medication effects can be found. This study proposes two different prediction models of proven significance and accuracy.

4.1 Immune Efficiency Test (IET)

Depending on the patient's anamnesis, ten to twenty different drugs are tested per IET. The motivation of each test is to identify drugs that positively affect the immune system and therefore promote the reduction of cancer cell proliferation. In this study, criteria for accurate medication were understood and mathematically described (positive drug effects are characterised by a negative *Induced Lymphocyte Activity;* see equation 1 in *Theoretical Fundamentals*). Drugs associated with an increase in cancer cell proliferation are excluded from the therapeutic regimen. At the time this study started, IET had been applied to 82 patients. Some patients were tested more than once. Altogether 56 different drugs had been tested resulting in more than 1200 single experiments.

4.2 IET Relational Database

In order to store, access and update general, clinical and IET data, a relational database was designed and implemented. Database design was performed within the three phases conceptual, logical and physical database design. The database was subsequently implemented on a MYSQL 4.0.23 relational database management system.

Conceptual Database Design. The dataset given hold general and medical patient information along with test data and results of the respective drugs. This results in the definition of five basic entities: *Patient*, *Immune Efficiency Test*, *Laboratory Tests*, *Diagnosis* and *Drug* (illustrated in Figure 11). All attributes of the initial dataset can be assigned to one of these entities.

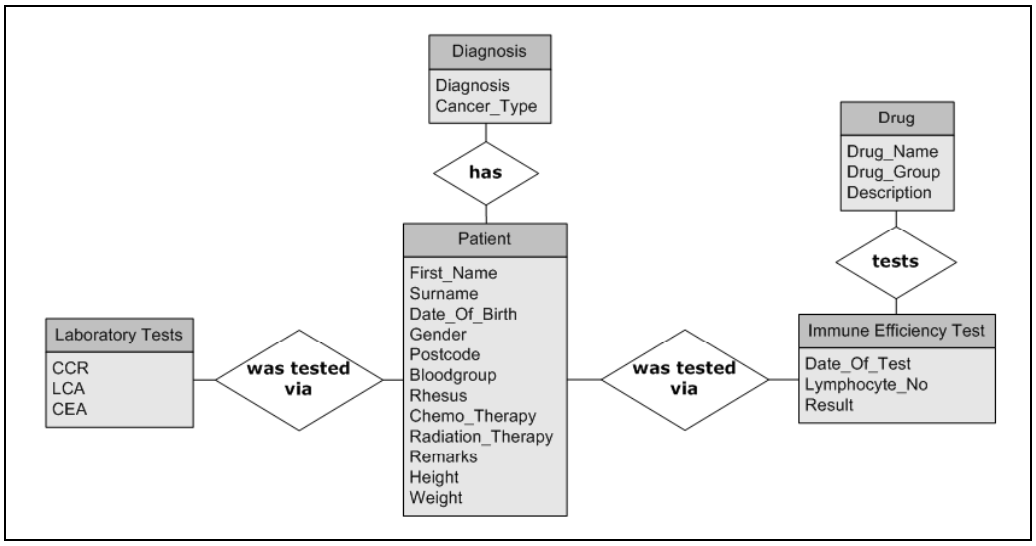
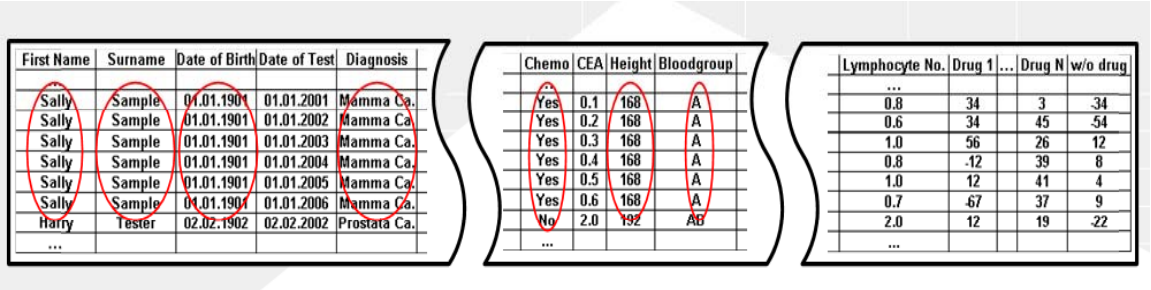


Figure 11 Entity Relationship (E/R) Model. The five basic entities *Patient*, *Immune Efficiency Test*, *Laboratory Tests*, *Drug* and *Diagnosis* are connected as shown above. All attributes of the dataset given can be assigned to one of the four entities as illustrated.

Logical Database Design. Logical database design develops a logical model describing the real world domain captured within the E/R model. For this purpose, normalisation of the initial dataset was performed.



First Name	Surname	Date of Birth	Date of Test	Diagnosis
...				
Sally	Sample	01.01.1901	01.01.2001	Mamma Ca.
Sally	Sample	01.01.1901	01.01.2002	Mamma Ca.
Sally	Sample	01.01.1901	01.01.2003	Mamma Ca.
Sally	Sample	01.01.1901	01.01.2004	Mamma Ca.
Sally	Sample	01.01.1901	01.01.2005	Mamma Ca.
Sally	Sample	01.01.1901	01.01.2006	Mamma Ca.
Harry	Tester	02.02.1902	02.02.2002	Prostata Ca.
...				

Chemo	CEA	Height	Bloodgroup
...			
Yes	0.1	168	A
Yes	0.2	168	A
Yes	0.3	168	A
Yes	0.4	168	A
Yes	0.5	168	A
Yes	0.6	168	A
No	2.0	192	AB
...			

Lymphocyte No.	Drug 1	...	Drug N	w/o drug
...				
0.8	34		3	-34
0.6	34		45	-54
1.0	56		26	12
0.8	-12		39	8
1.0	12		41	4
0.7	-67		37	9
2.0	12		19	-22
...				

Figure 12 Un-normalised Form (UNF) of the IET database. The UNF contained redundant information (highlighted red) that was removed during the process of normalisation.

Figure 12 reveals some of the dependencies that occurred in the dataset given. Test information depends on patient information in a way so that each patient can have more than one single test, but each test requires exactly one single patient. Moreover, each patient needs to have one single diagnosis, whereas the same diagnosis can be assigned to more than one patient. In addition, each single drug has metadata including a drug group and a description, but each drug group may describe more than one drug. Therefore, redundancies occur within the UNF. In order to transfer the UNF into 1NF all redundancies had to be resolved.

For this purpose, separate patient and test tables along with the unique identifiers *Patient_ID* and *Test_ID* were created. In addition, both tables were further split in order to resolve remaining redundancies and to integrate metadata (drug group and description). This was done by generating *Diagnosis* and *Drug* tables. Consequently, this required a separate *IET_Results* table for assessing the test results of each drug being tested within a particular IET. *IET_Results* is a so-called "weak entity"[2], which is only defined by the two tables *Drug* and *Test*. After creating the unique identifiers *Diagnosis_ID* and *Drug_ID* for the tables *Diagnosis* and *Drug*, further investigation of these five tables revealed that the design was also in 2NF, since no partial dependencies occurred.

[2] In relational databases, a weak entity is an entity that cannot be uniquely identified by its own attributes alone (http://en.wikipedia.org/wiki/Weak_entity).

To simplify querying of the database and to match database design with the E/R model, the *Test* and *Patient* tables were further split by generating separate *Lab_Results* and *Anamnesis* tables.

In order to transfer the design into 3NF, all transitive dependencies had to be resolved. Transitive dependencies occurred within the *Diagnosis* and *Drug* tables. The *Cancer_Type* and *Drug_Group* attributes are transitively dependent on *Diagnosis and Drug_Name,* respectively. However, during design of the database, it was found to be more efficient keeping this part of the database in 2NF, since its removal would complicate queries and therefore not increase the efficiency of the database in any significant way. The entire normalisation process starting with the UNF is summarised in Figure 13.

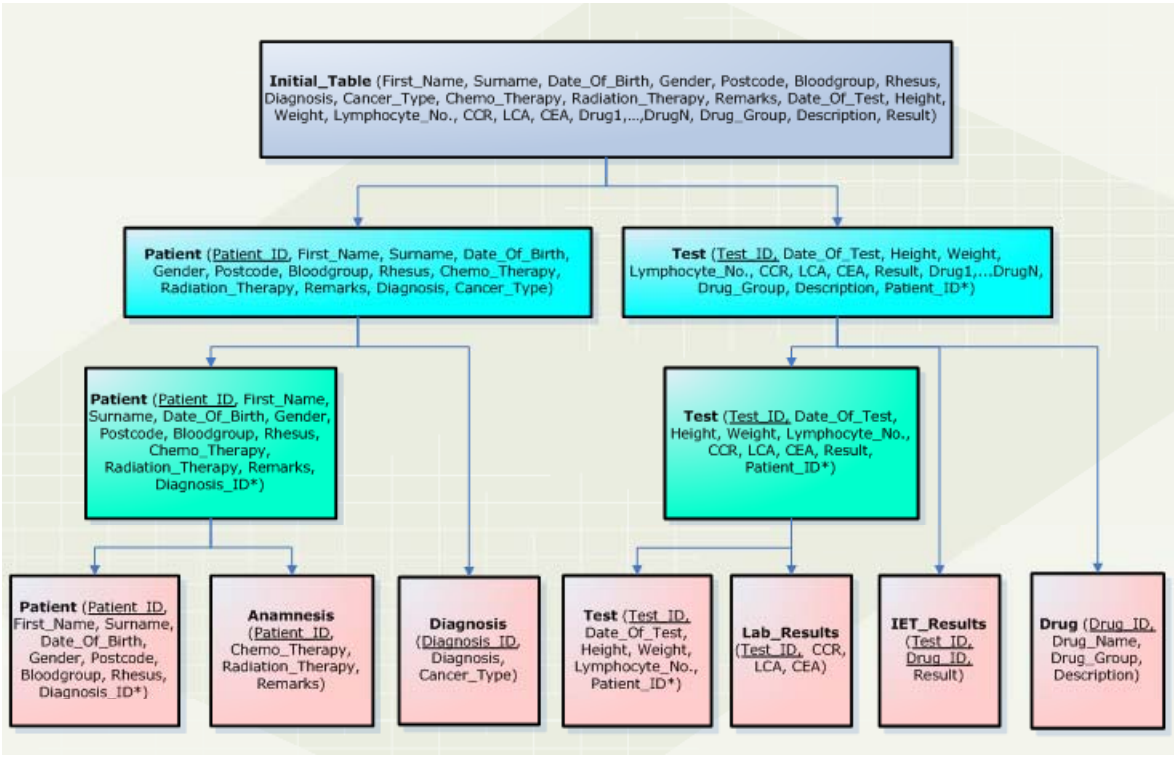


Figure 13 Normalisation Process. The UNF is labelled blue. The resulting two *Patient* and *Test* tables (light blue) constitute intermediate formats being subsequently transferred into 1NF (green). Further splitting of the *Patient* and *Test* tables, simplifies querying and reflects the E/R model. The resulting structure is in 2NF (red). Primary keys are underlined and foreign keys are labelled with "*".

The resulting database design, stores IET data in the *Test*, *IET_Results* and *Drug* tables. The *Patient*, *Anamnesis*, *Diagnosis* and *Lab_Results* tables maintain general and clinical information. The tables and their

relationships are summarised in the logical model presented in Figure 14.

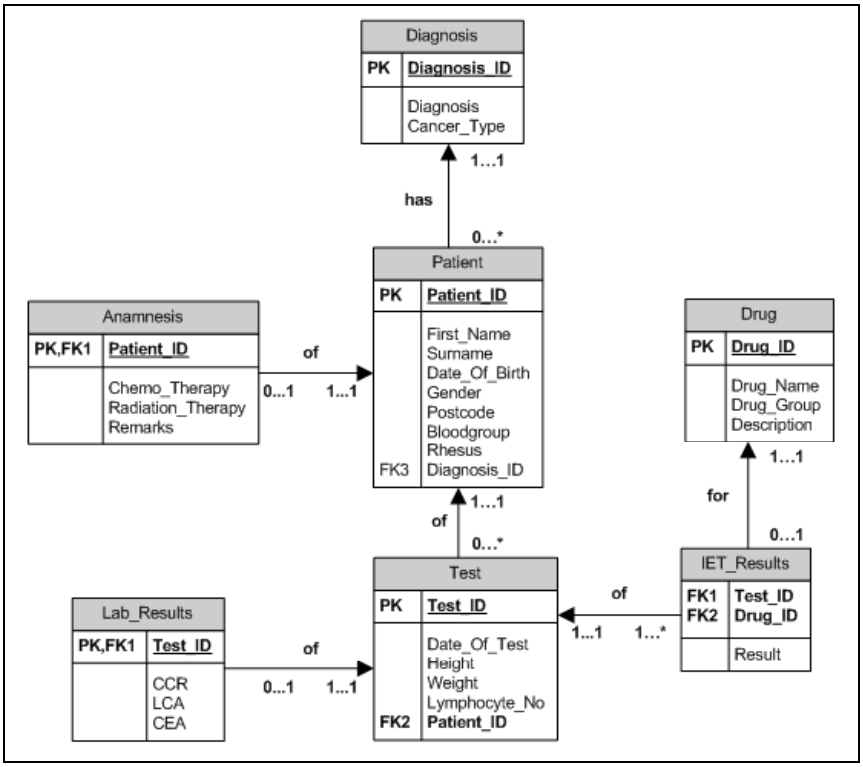


Figure 14 Relational Model of the IET-Database in 2NF. The relational model shows tables, relationships and attributes. The different tables are assigned to the three types of information stored within the database: general, clinical and IET information. Primary keys (PK) are underlined and 'FK' indicates foreign keys. The arrows indicate the direction of the dependency. For instance, *Anamnesis* depends on *Patient*.

Physical Database Design. The relational model illustrated in Figure 14 served as a logical model for physical database design. The tables of the relational model were directly used to implement the database. All attributes, primary and foreign keys as illustrated in Figure 14 could be adopted to implement the database on a relational database management system. The manageable size of the database, did not require disk space and performance considerations so far.

4.3 IET – Web Interface

In order to maintain, update and access the database, a user-friendly web interface was developed and connected to the IETDB using three Tier architecture. Here, the IET-web interface constitutes the top tier and provides user interference. The third tier provides IETDB management functionality and is implemented using a MySQL database

server. The middle tier provides process management services that can be shared by multiple applications. Here, an Apache/2.0.48 web server was employed.

This reflects the fact that the database will not only serve as a local data repository, but might be accessed via the internet in the near future. For this reason, a basic user management model was developed. The web interface distinguishes between 'read only' and 'read/write' users.

The IET - web interface operates the underlying database and offers three main functionalities for user interaction:

(1) **Data Retrieval –** Information stored in the database can be displayed and optionally be downloaded.

(2) **Data Insertion –** New information can be incorporated into the database.

(3) **Data Editing** – Information stored in the database can be edited.

Data Retrieval – Two basically different options for data retrieval were implemented in the IET web interface. The first involves a pure display of information. This includes general drug and patient details, the respective IET and laboratory results, along with information about diagnosis and anamnesis. This option will primarily be used by medical practitioners requiring a platform for easy and fast information retrieval, for example details concerning a particular patient.

In addition to this display function, the interface provides a download option to retrieve the entire database as a comma separated value (csv) file. In addition, a documentation file for the database can be displayed and alternatively be downloaded (*IET Documentation* function). This functionality was implemented for analysts who for example perform data mining on the database. Both functionalities are shown in Figure 15.

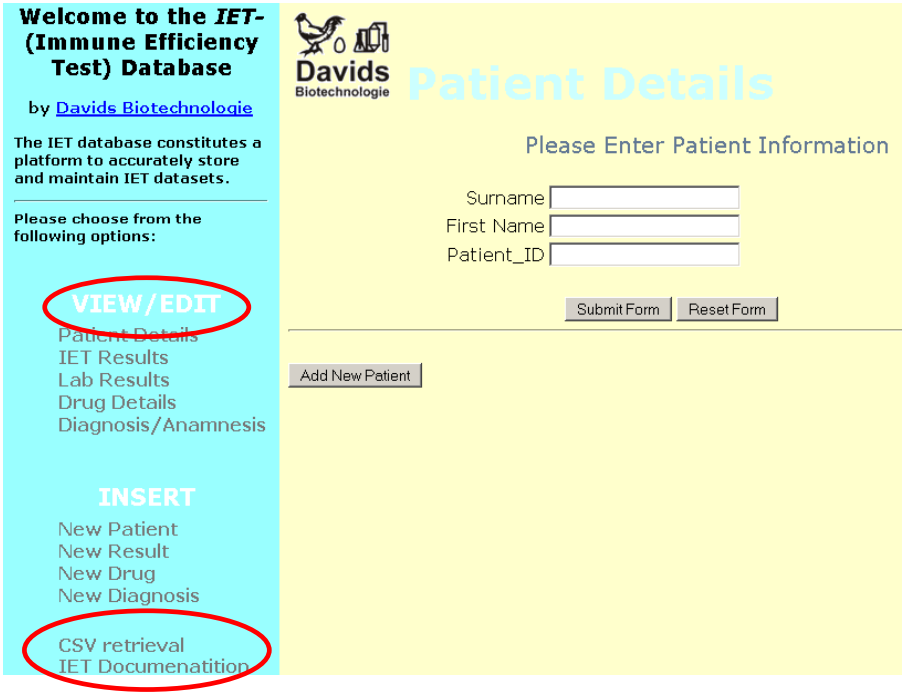


Figure 15 Data Retrieval. Two distinct modes for data retrieval were developed: a pure display of information accessible via the *VIEW* functions (upper red circle) and the *CSV retrieval* and *IET Documentation* (lower red circle) download options. The right frame shows the retrieval form used to access patient details.

Data Insertion – The insert option was developed for medical practitioners and can only be accessed by read/write users. This function supports incorporation of new drug-, diagnosis- and patient-information along with the respective IET- and laboratory results. Figure 16 illustrates how to insert new patient information.

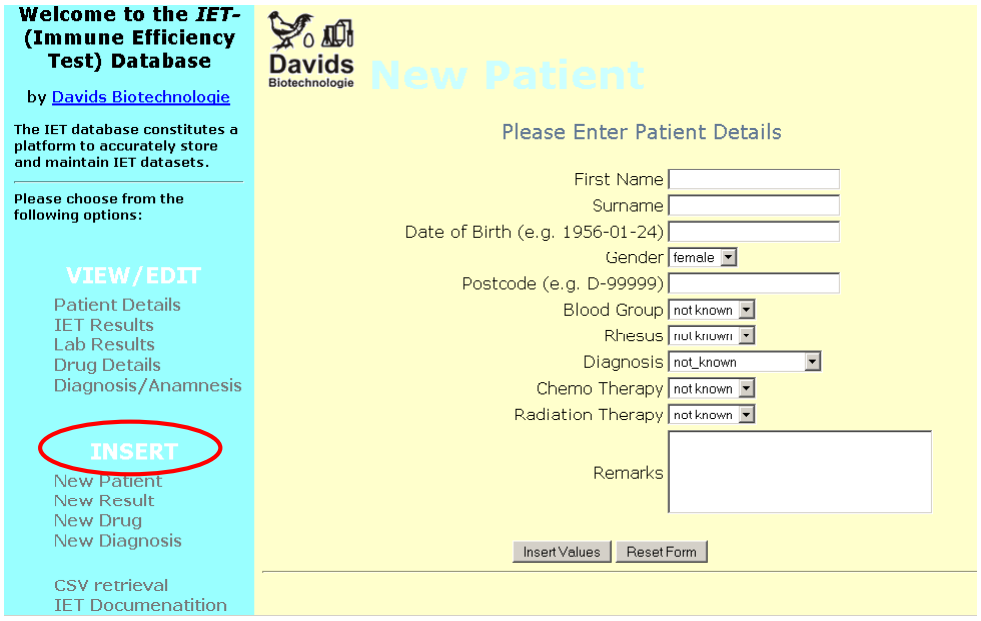


Figure 16 Data Insertion. Four options were developed for data insertion, namely *New Patient*, *New Result*, *New Drug* and *New Diagnosis*. Exemplarily, insertion of new patient information is displayed in the right frame.

The *New Results* option operates both the insertion of new IET data and laboratory information. New results can only be inserted if the patient already exists within the database. Insertion of new results is performed in two steps. First, general and clinical information is entered and the drugs being tested are selected from a pull-down list. Second, IET results are entered for the respective drugs. These two steps are linked via confirmation logic implemented by embedded JavaScript code that summarises all inputs of the first step (Figure 17). This aims to prevent the aberrant storage of type errors into the database. Another JavaScript pop-up window has to be confirmed before IET results are sent to the database (this is not shown in Figure 17). All other *Insert* options were linked to JavaScript confirmation logic in the same way.

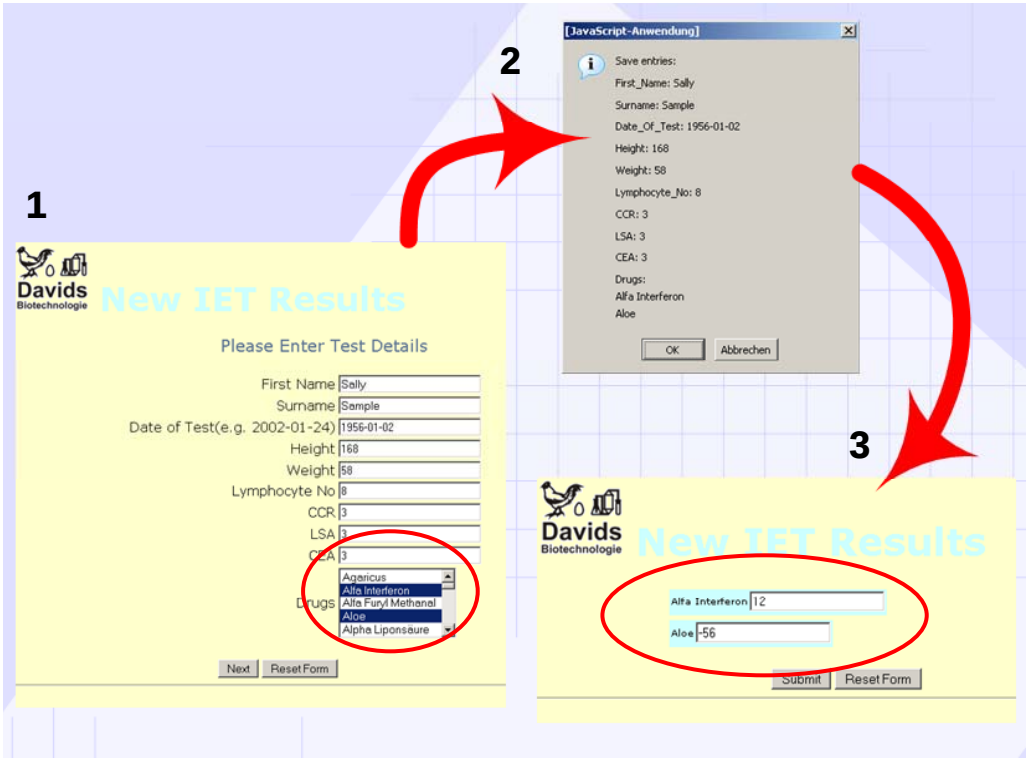


Figure 17 Data Insertion – New Result. Insertion of new results (IET and laboratory results) is carried out in two steps. First, the insertion of general information such as *Height* and *Lymphocyte No* plus the selection of the drugs being tested (left red circle, 1). Second, the insertion of the IET results for the previously selected drugs (right red circle, 3). These two steps were linked via a JavaScript pop-up window (2) to confirm the entries of the first step. Before sending the new results to the database, another JavaScript pop-up window needs to be confirmed (not shown in Figure 17).

Data Editing – Data editing is closely linked to data retrieval and uses the *VIEW* options to select the type of information for being edited or deleted. This functionality can only be accessed by read/write users. The result frame of each query provides options for editing or deleting. In case the *delete record* option was chosen, deletion needs to be confirmed before the command will be executed at the database level. The *edit record* option leads to another frame to change the respective entry. The edition of the entry needs to be confirmed in a java-script pop-up window. Figure 18 shows this sequence of forms for updating patient information.

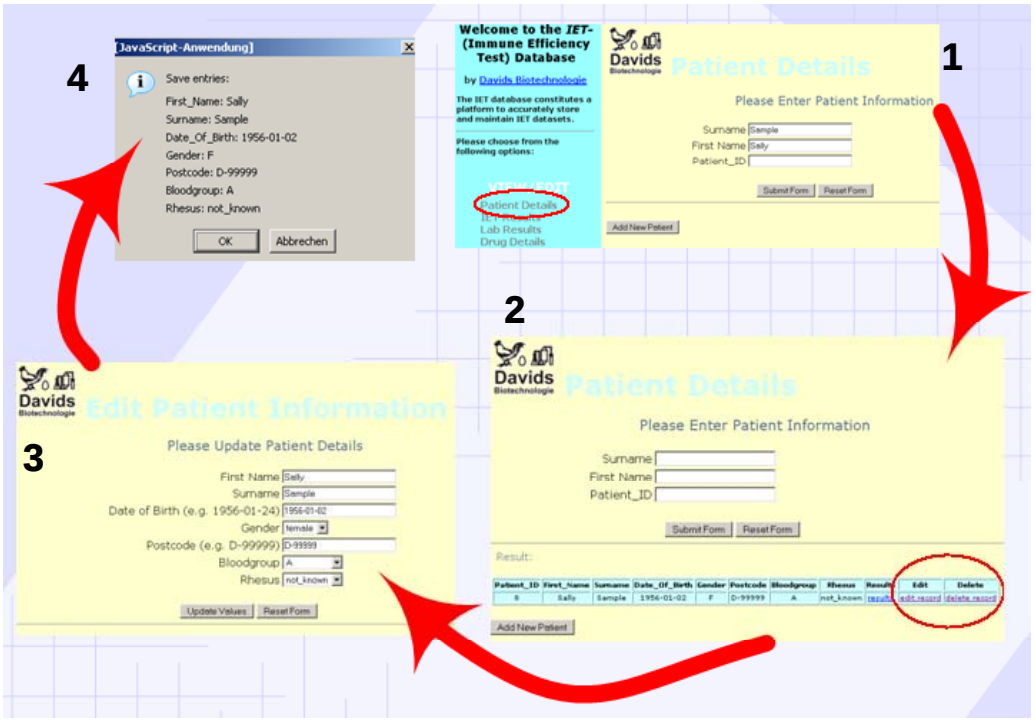


Figure 18 Edit Data. Editing of records is shown for patient information. After querying for a particular patient (1), the result frame (2) gives options to edit or delete the respective entry. When selecting *edit record* the *Edit Patient Information* menu (3) is started. Before updating the database with the edited version of the record, all alterations must be confirmed in a JavaScript pop-up window (4).

In conclusion, the IET web interface provides standard functionalities to maintain and update the database, and to view and retrieve the information stored. Since the database holds sensitive patient information, the following three aspects of data security were implemented:

(1) **Secure Data Transfer Protocols (SSL) –** Secure Sockets Layer (SSL) is a asymmetrical cryptographic protocol providing secure communication and endpoint authentication on the internet. The Apache server used was authenticated using SSL 3.0.

(2) **Validation of Potentially Insecure User Input -** PHP provides the function *mysql_real_escape_string()* to escape potentially insecure user input and therefore to avoid SQL Injections. The term SQL Injection defines a security vulnerability occurring in the database layer of an application by incorrect escaping of string literals embedded in SQL.

(3) **Basic Access Management –** User management of the IETDB web interface covers two different user situations: read only users and read/write users. For both password-protection was implemented.

4.4 Data Mining

The fundamental objective of this data mining study was to investigate whether or not predictive models estimating future effects of particular drugs on individual patients can be built and validated based on IET data.

Therefore, this study had access to more than 80 immune efficiency tests (~ 1200 single measurements). Each measurement constituted an individual experiment investigating the ability of lymphocytes to eliminate cancer cells under the influence of particular drugs. Here, the proliferation rate of the cancer cells was monitored. Along with the respective clinical and general patient information, individual *immune efficiency profiles* were designed. These profiles can be used in data mining, for instance, to discover biologically similar drugs or risk circumstances for cancer development or to construct multivariate predictors of group memberships. The latter is covered within this study which proposes two models for predicting the effect of particular drugs

on cancer patients. Here, principles of CRSIP-DM methodology (Chapman 1999) are applied.

4.4.1 Business understanding

Business Objectives. This data mining project integrated IET, general and clinical information resulting in the generation of individual *immune efficiency profiles*. The project's objective was to clarify whether or not prediction models for personalised medication can be developed based on the exploration of immune efficiency profiles.

Assess situation. All resources required to achieve the business objective were available. These included CRISP-DM 1.0 data mining guide, Clementine Graduate Pack 8.1 and the dataset itself, which could legally be used for this study. Recommended system requirements for Clementine were fulfilled (*Operating System:* Microsoft Windows XP or Windows 2000 Professional; *Hardware:* Intel Pentium compatible processor; *Memory:* 512 MB RAM; Minimum free disk space: 320MB (SPSS 2005)).

Data mining objectives. In order to implement the *business objective*, the following four *data mining objectives* needed to be achieved.

(1) **Data Integration –** Combination of general, clinical and IET data in order to design individual immune efficiency profiles.

(2) **Test for Attribute Dependency –** The detection of statistical attribute dependency that indicates underlying patterns within the profiles and makes model building reasonable.

(3) **Model Building -** Design of prediction models for personalised medication.

(4) **Quality and Significance Assessment –** Validation of the prediction models designed.

Project Plan. The project schedule of this data mining project was basically structured by CRISP-DM 1.0 methodology (compare Figure 8). All data available were used for model building and validation. Therefore, no data could be placed at the disposal of a deployment phase. For this reason, the deployment phase was excluded, although all other considerations of CRISP-DM were implemented in this project.

4.4.2 Data understanding

Data understanding results in advice and considerations that need to be conducted in the data pre-processing phase. Here, we can divide the considerations found into three categories, namely *Dataset*, *Data* and *Attribute considerations*.

Dataset Considerations – As a requirement of the third data mining objective (pp. 47), the class attribute of the dataset needs to be a measure representing the effect of particular drugs. In the initial dataset, this demand was fulfilled by the attributes *Drug1* to *Drug57*. In order to design prediction models for medication based on IET data, one single class attribute (***A**ctual **C**lass **L**abel*, ACL) is required. A statistical model predicts the ACL by generating ***P**redicted **C**lass **L**abels* (PCL). For that reason, the initial dataset needs to be transformed from a patient-wise into a drug-wise format. This implies the substitution of the patient-oriented attributes *Drug1* to *Drug57* by one drug-oriented class attribute representing drug effects and one additional attribute identifying the respective drug.

For model building and validation, the initial dataset needs to be divided into a training set and a validation set. To guarantee compatibility to all modelling algorithms of potential interest, the dataset needs to be formatted.

Data Considerations – In most cases, more than one test was performed per patient. Results of the test were used to adjust medication. However, information about these adjustments was not

available. For this reason, only the first test of each patient can be included, since subsequent tests do not guarantee independence.

Moreover, a detailed data quality report is required to assess if it is necessary to remove other records.

Attribute Considerations – Generally, attribute operations may increase knowledge and spur the achievement of data mining goals. In the given dataset, some of the drugs can be grouped based on their chemical, structural or functional properties. Therefore, it is reasonable to introduce a new attribute called *Drug Group*.

Moreover, the combination of attributes may increase the information content. In our case, the two attributes *Date of Test* and *Date of Birth* should be merged to the more meaningful attribute, *Age at Test*. The attributes *Height* and *Weight* should be linked to the attribute *BMI* (body mass index).

In addition, exclusion of attributes might be a useful means to increase the accuracy of prediction models. All attributes originating from database design, including *Test ID* and *Patient ID* can be excluded. Moreover, the attribute *Postcode* is of no relevance within this study. However, the importance of attributes can hardly be predicted before applying modelling algorithms to the dataset. Nevertheless, the impact of the attributes *Blood Group* and *Rhesus* to cancer was analysed more closely, since their influence in cancer is already well-reported (Dabelsteen 2005; Madjd 2005; Schneider 2005; Yei 2005). Several publications associate blood group effects with breast carcinoma, oral or gastric cancer (Dabelsteen 2005; Madjd 2005; Yei 2005). Moreover, in 2005, Schneider et al proved the significant impact of the rhesus factor on the development of symptomatic meningioma and found that rhesus positive cases were less frequent in the underlying patient group (Schneider 2005). Basal analyses of the IET dataset showed strong interactions of blood group A and status malignant (60.66%, Figure 19).

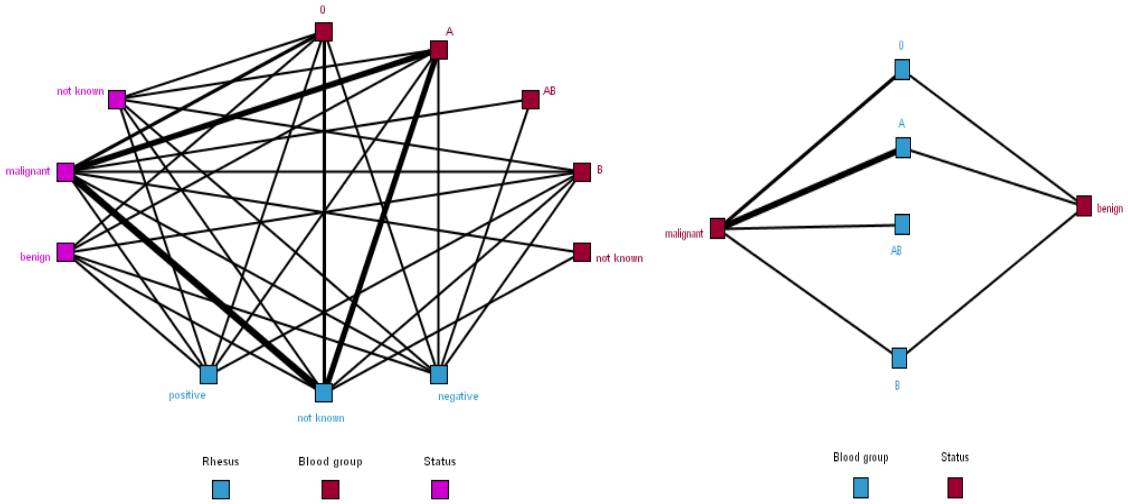


Figure 19 Web Graphs of the Attributes *Blood Group*, *Rhesus* and *Status*. Strong interactions were shown for blood group A, malignant and unknown rhesus factor (left graph, strong interactions are labelled bold). When focusing on the attributes *Blood Group* and *Status* (right graph) the correlation between blood group A and the malignant state became more evident. No conclusion could be drawn from interactions between blood group AB and the malignant state, since blood group AB is only represented by one single record. Significant associations between blood group 0 and either malignant (27.27%) or benign (24.59%) were not found.

Since blood group AB was only represented by one single record, associations of blood group AB could not be taken into account. The attribute *Rhesus* may be removed from the dataset due to its high number of missing values (69.51%).

In conclusion, strong associations were only found between blood group A and the malignant state, whereas blood group 0 and B were characterised by indifferent interactions. Consequently, the attribute *Blood Group* needs to be included in this study. No associations of the attribute *Rhesus* were found due to low data quality. This shows the need for general attribute quality verification.

Conclusions. In Table 4, all suggestions and considerations obtained from the *Data understanding* phase are summarised for being transformed into operations within the subsequent data pre-processing phase.

Dataset Considerations	Data Considerations	Attribute Considerations
Dataset Transformation: Transform the dataset from a patient-wise into a drug-wise format; this includes some attribute operations.	**Series Exclusion:** Exclude test series by only selecting the first test of each patient; this guarantees independence.	**Grouping:** Group the various drugs by introducing the new attribute, *Drug Group.*
Formatting: Before starting model building, the final dataset needs to be formatted to guarantee compatibility with modelling algorithms.	**Data Quality Analysis:** Decide whether or not particular entries should be excluded.	**Combination:** Combine the following attributes: *Date of Test*/*Date of Birth* to *Age at Test* and *Weight*/*Height* to *BMI.*
Sampling: Generation of training and validation subsets for evaluation.		**Exclusion:** Analyse attribute quality and relevance.

Table 4 Advices and Consideration Acquired from Data Exploration.

4.4.3 Data pre-processing

In this study, data pre-processing was mainly concerned with the integration of general, clinical and proliferation information. This provided a suitable dataset of individual immune efficiency profiles for data mining. All considerations derived from the data understanding phase were transformed into operations and finally aided in obtaining reliable prediction models for the data.

Dataset Operations

Dataset Transformation. To perform the transformation from a patient-wise into a drug-wise format, a java application (Transformer.java) was developed. Functionality of this application is illustrated in Figure 20.

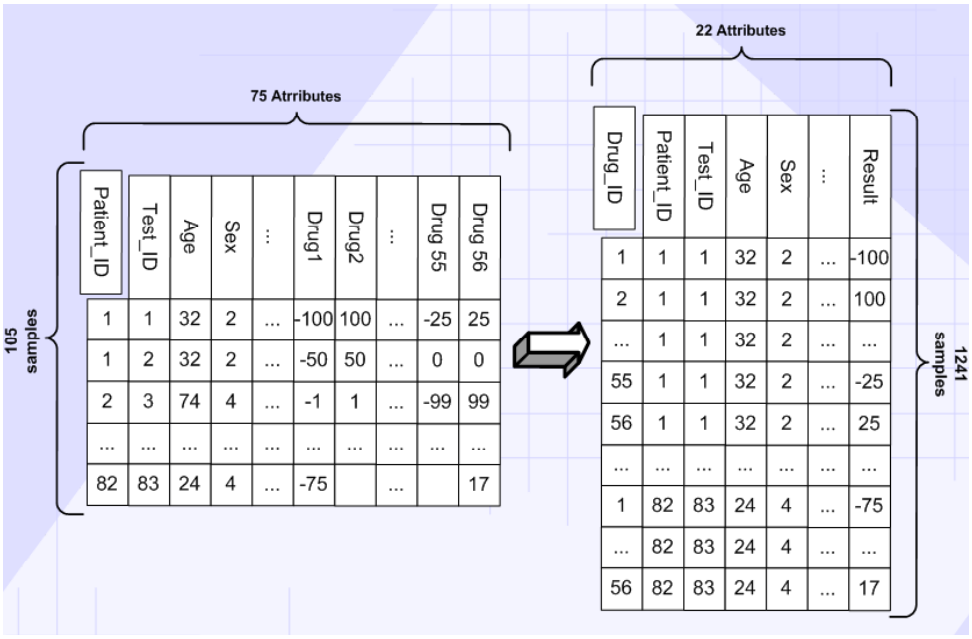


Figure 20 Dataset Transformation from Patient-wise to Drug-wise Format. This operation was performed by the java application Transformer.java.

Here, the initial attributes *Drug 1* to *Drug 56* were substituted by the two attributes *Result* and *Drug ID*. The attribute *w/o Drug* got the *Drug_ID* "0". All other drug IDs (e.g. "3") represented the initial attribute name (e.g. Drug 3). This guaranteed unique identification of each particular proliferation measurement and defined the single class attribute *Result*. In doing so, the number of attributes decreased from 75 to 22 while the number of instances increased from 106 to 1241.

Sampling. Another java application (RandomDistributor.java) was developed to generate random subsets of the initial dataset. These subsets were required for training and validation of prediction models. The training set hold ~70% (871 instances) and the validation set ~30% (370 instances) of the initial dataset. Here, it was crucial to guarantee that the two datasets did not overlap and that the validation set was not used in any way for model building.

Data Operations

Series Exclusion. Only the first test of each patient was taken for further investigation. Exclusion of the remaining entries was performed within the original excel file before transferring the dataset into Clementine.

Data Quality Analysis. In Clementine, a *Quality* node was used to assess data quality. Missing values needed to be declared in Clementine but did not affect further modelling. This was done by using a *Type* node.

Attribute Operations

Grouping. Most drugs could be grouped according to their physical, biological or chemical properties as shown in Table 5. For that reason, a new attribute *Drug Group* was included, without substituting the attribute *Drug ID*. This operation was also implemented in MS Excel by using basic if/else expressions.

Group	Drug ID	Description
Group 0	0	Control
Group 1	37, 41, 50, 56	Enzyme
Group 2	13, 18, 22, 25, 26	Mistletoe
Group 3	32, 33, 47, 48, 49	Thymus
Group 4	30, 54, 55	Vitamin
Group 5	All others	Others

Table 5 Grouping of *Drug ID* According to Chemical or Structural properties. This table lists all drug subgroups and their respective candidates. Due to data security, the actual drug can not be published yet. Please note that *Drug ID* "0" represents the control reactions and is listed as a separate group.

Combination. In this study, the attributes *Date of Birth* and *Date of Test* were less important than the age of a particular patient. Therefore, *Date of Birth* and *Date of Test* were merged to a new attribute called *Age*. The attributes *Height* and *Weight* were combined in the same way by introducing a new attribute called *BMI* (body mass index). This reduced feature space and simplified the initial dataset without affecting the amount of information. This operation was undertaken in Clementine.

Exclusion. Criteria for data selection include relevance of information for the data mining objectives and data quality. As discussed before, the attributes *Postcode*, *Patient ID* and *Test ID* did not contribute to knowledge discovery and were therefore omitted. The 17 remaining attributes, were analysed in terms of data quality. This included two tasks. First, the declaration of all user defined missing values and second, the quality analysis and exclusion of attributes characterised by low data quality. Missing values have already been declared in *Data Operations*. Results of the data quality analysis are listed below. Both data quality analysis and attribute exclusion were performed by using a Quality node which excludes values of quality lower than 50% by default. Table 6 shows data quality of the various attributes and indicates those being excluded for further investigations in bold.

Attribute	% complete	Number of Legal values
Status	92.1	1143
Sex	100.0	1241
Age	94.2	1169
Diagnosis	92.1	1143
BlastELSEmic layer	92.1	1143
Chemo Therapy	**39.24**	487
Radiation Therapy	**39.24**	487
CCR	**10.64**	132
LSA	**5.48**	68
CEA	**6.77**	84
BMI	100.0	1241
Blood Group	99.03	1229
Rhesus	**33.36**	414
Lymphocyte No.	100.0	1241
Drug ID	100.0	1241
Drug Group	100.0	1241
Result	100.0	1241

Table 6 Data Quality Report. Completion rate and the number of legal values are provided for each attribute. Attributes being excluded after assessing data quality are labelled in bold.

Format Data

To guarantee compatibility with the modelling algorithms used, the dataset needed to be formatted. Most machine learning algorithms require discrete class labels. This demanded the introduction of the new class attribute *Effect* based on the former class attribute *Result*. All *Result* values were assigned to one of the following subsets: *high increase*, *increase*, *stagnancy*, *reduction* and *high reduction* of the proliferation rate. Identification of the various subsets was done by applying a scheme of even numbers '0', '2', '4', '6', '8' as summarised in Table 7.

Change of Proliferation rate [%]	Effect	Explanation
-10 to 10	0	Stagnancy
10 to 50	2	Increase
-10 to -50	4	Reduction
50 to 100	6	High Increase
-50 to -100	8	High Reduction

Table 7 Relationship of the Proliferation Rate and the New Class Attribute *Effect.* Based on the change of the proliferation rate (-100% to 100%), the new class attribute *Effect* was designed. Here, a scheme of even numbers was applied, but other schemes may serve equally.

Here, reduction of the proliferation rate indicated effectiveness of the respective drug, since proliferation of the cancer cell line was reduced. In turn, *Increase* signalled an ineffective drug, since the growth of the cancer cell line was up-regulated.

Most commonly, numeric variables are required for analysis. Therefore, the storage type of all string attributes was transformed to numeric. This affects the *Status*, *Sex*, *Blastodermic Layer*, *Diagnosis* and *Blood Group* attributes. These string attributes were transformed by a scheme of even numbers as listed in Figure 21.

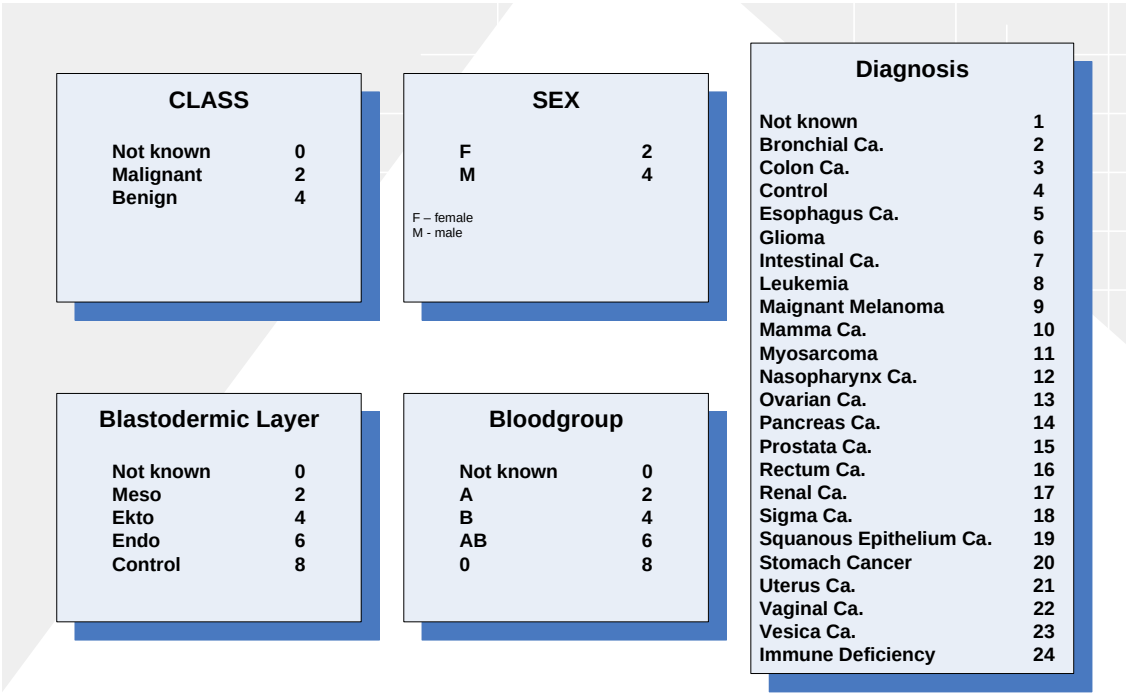


Figure 21 Compilation of String and Numeric Values of the Respective Attributes. Attributes of the storage type string were transformed into numeric values. Figure 21 shows the initial string value in contrast to the new numeric value.

Please note that cases of immune deficiency (ID 24) were treated separately from control cases, although both attributes are classified as benign. Cancer patients frequently show immune deficiency as well and therefore it is essential to distinguish between these cases. Otherwise discrimination of control and cancer cases may decrease significantly.

In conclusion, the sequence of operations described above produced two datasets (training and validation set) of 11 attributes holding 1241 records altogether. Table 8 summarises the attributes being used for modelling, reports completion rates and lists the range of values, as well as storage and data types.

Attribute	% complete	Storage Type	Data Type	Value
Age	94.2	Real	Range	[22.5, 91.4]
Blastodermic layer	92.1	Integer	Set	0*, 2, 4, 6, 8
Blood Group	99.03	Integer	Set	0*, 2, 4, 6, 8
BMI	100.0	Real	Range	[16.7, 36.7]
Diagnosis ID	92.1	Integer	Set	1,…,24
Drug Group	100.0	Integer	Set	0, 1, 2, 3, 4, 5
Drug ID	100.0	Integer	Set	0,…,56
Lymphocyte No.	100.0	1Real	Range	[0.31, 4.5]
Sex	100.0	Integer	Flag	2, 4
Status	92.1	Integer	Set	0*, 2, 4
Effect	**100.0**	**Integer**	**Set**	**0, 2, 4, 6, 8**

Table 8 Final Attribute Properties. Completion rates, the range of values, as well as data and storage types are listed for each of the 11 attributes of the final dataset. The class attribute *Effect* is in bold. Values labelled with * constitute user defined missing values and once declared, they do not appear in Clementine any more.

The dimensionality of the feature space was reduced from 75 to 11. Most importantly, the dataset was transformed into a drug-wise format integrating clinical, general and proliferation information. The combination of these different sources of information resulted in individual ***immune efficiency profiles***, which can be used for testing whether or not prediction models for the class attribute *Effect* can be built.

4.4.4 Modelling

In order to answer the question whether or not prediction models based on *immune efficiency profiles* can be built, four tasks were to be executed.

(1) Hypothesis Testing for Statistical Dependencies

(2) Determine Settings for Class Prediction

(3) Selection of the Prediction Method

(4) Model Building

Hypothesis Testing for Statistical Dependencies. Prior to starting the model building process, it was essential to probe whether or not statistical dependencies of the attributes can be found. In the case of statistical dependency, we can assume underlying patterns within the

dataset. It is only then that further investigations to determine prediction models become reasonable.

For this exercise, LIAN 3.0 (Haubold 1998; Haubold 2000) was used. Analyses of the dataset using LIAN showed highly significant associations between attributes ($P = 10^{-4}$) and therefore gave reason to suspect patterns associated with the dataset. Based on this finding, modelling algorithms were applied to the dataset to build meaningful prediction models.

Settings for Class Prediction. This study aimed to produce prediction models for the declared class attribute *Effect.* For this reason, supervised algorithms were most useful for model building.

Selection of a Prediction Method. In this study, a prediction method loosely based on Dudoit and colleges (Dudoit 2002) was applied by employing the two modelling algorithms *ANN* and *CART*.

Model Building. C&R tree, a CART version of Clementine, and an ANN approach were applied for model building. The C&R tree algorithm (using default settings with a maximum tree depth of 10) applied to the training dataset, identified the attributes *Diagnosis ID* and *Lymphocyte No* as most important for class prediction. The ANN algorithm using default settings without the prevention of overtraining, defined the attributes *Blood Group*, *Blastodermic Layer*, *Age* and *Lymphocyte No* as most important. These attributes are characterised by their similar relative importance (0.19, 0.18, 0,14 and 0.13).

4.4.5 Evaluation

Regardless of the prediction method chosen, in order to assess the quality and significance of the models, it was necessary to perform validation and randomisation testing, asking the question "if the models perform well on the training set, will they also predict the class attribute accurately when applied on unknown data?"

When testing the two models on the validation set, the *C&R tree* model achieved an accuracy of 63.0%. In 61.1% of the cases, the *ANN* model predicted the class attribute correctly. These findings showed that the two algorithms can be used to build predictive models estimating the effect of a specific drug, based on immune efficiency profiles. However, significance of the models has not yet been proven. For that reason, randomisation testing was performed. This task was conducted by developing a java application (RandomisationTesting.java). Randomisation testing showed that correct class prediction by chance alone obtained a mean accuracy of 34% (Figure 22) and a p-value of $1x10^{-5}$ when compared to the two prediction models.

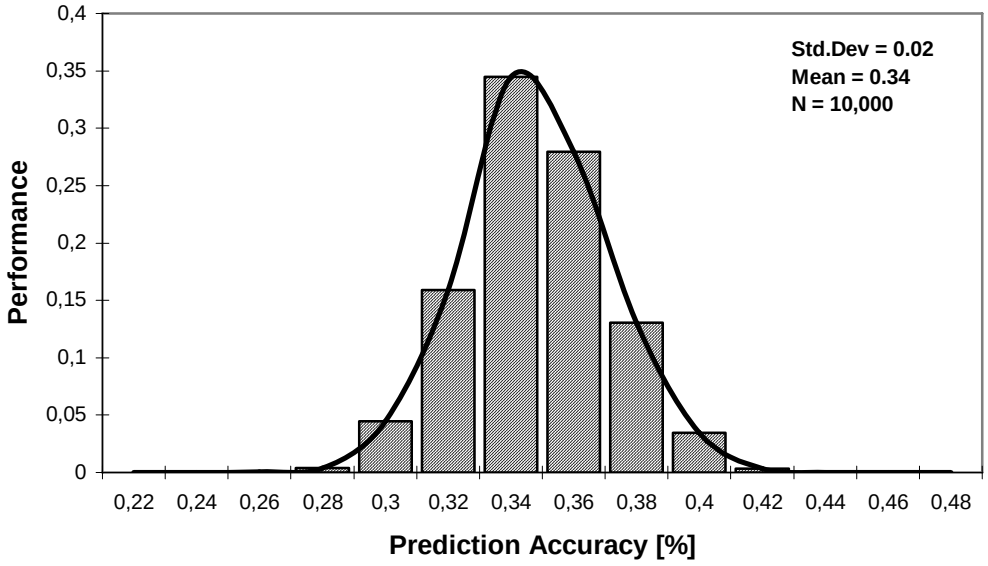


Figure 22 Randomisation Test. Distribution of prediction accuracy produced by N = 10,000 randomly generated predictions.

This evidence definitely reveals the significance of the previously designed models.

In conclusion, comprehensive and coherent immune efficiency profiles were generated. Attribute dependency was proven and based on this finding, machine learning algorithms were applied to the dataset in order to build prediction models. Finally, the models generated were validated and significance was assessed via randomisation testing. The phases, tasks and tools employed to achieve the data mining objectives of this project are briefly summarised in Table 9.

Data Mining Objectives	CRISP-DM Phases	Operations	Tools
Data Integration	Data Pre-processing	Dataset Operations	ColumnSwapper.java Randomiser.java
		Data Operations	Excel/Clementine
		Attribute Operations	Excel/Clementine
		Format Data	Clementine
Attribute Dependency	Modelling	Hypothesis testing for statistical dependencies	LIAN 3.0
Model Building	Modelling	C&R tree	Clementine
		ANN	Clementine
Quality Assessment	Evaluation	Validation	Clementine
		Randomisation Testing	RandomisationTester.java

Table 9 Data Mining IET Data. Summary of tasks and tools applied to achieve the respective data mining objectives during the various CRISP-DM phases.

These findings show that based on the initial dataset, significant prediction models for medication can be built. Thus, the business, and consequently all data mining objectives, were achieved.

5 Discussion

Immune Efficiency Test – The main focus of conventional cancer therapy including chemo- and radiation therapy is to maximise tumour eradication within the limits of tolerable toxicity and radiation to the organism. Recently, several approaches arose to overcome or assist such conventional therapy. These include adjuvant and neo-adjuvant therapy, immunotherapy and theranostic approaches. Within the field of theranostics, the immune efficiency test (IET) presented in this study provides a novel approach of individualising cancer therapy as an alternative or supplement to conventional therapy. The IET combines immunotherapy with theranostics and enables the medical practitioner to selectively compare different treatment strategies and to optimise the patients' therapeutic regimen based on these findings.

Storage and Maintenance of IET data – This thesis presents techniques required to store and maintain IET data, along with supplemental patient information including general and clinical data. For this purpose, a relational database was created. To access this data, a web interface was implemented and connected to the database. The database efficiently represents the "real world" domain. However, future alterations concerning the IET and/or supplemental information may require adjustments of the database. Similarly, alterations of the web interface may also be required once the system is deployed and tested. This study identified and implemented two different user types – *read only users* and *read/write users.* This results in a clearly defined permission management for accessing the functionalities for data retrieval, data editing and deletion. This is sufficient to effectively access and maintain the current database. However, database growth and accessibility of the database via the Web and/or a local network, may require adjustments of user management, functionalities and security aspects.

Analysis of IET data – In 1999, Shochat and colleges presented a computational approach using "…computer simulations for evaluating the efficacy of breast cancer chemotherapy protocols" (Shochat 1999). Schochat modelled the effect of chemotherapy on tumour mass and simulated the outcome of several treatment protocols. The ensuing mathematical models have been used to design treatment protocols and to identify patterns among the many combinations of drug dosage and scheduling. Their work showed that computer sciences may enhance medical research and help to individualise therapy. In recent years, several other research groups demonstrated the utility of the concept of computer aided medical research and therapy. In 2005, Arakelyan et al. validated a computer model for predicting tumour growth based on data obtained by dynamic measurements of tumour growth and vascularisation dynamics (Arakelyan 2005). In addition, computer modelling has been used to simulate anaphylactic reactions due to chemotherapeutic reactions (Castiglione 2003) and to understand the complex process of angiogenesis – the formation of new blood vessels (Arakelyan 2003) – which has a widespread significance in cancer therapy (Folkman 1995; Folkman 2005).

Within this context, this thesis combines the concept of theranostics in immunotherapy with computer science. This study proves that the modelling of IET data provides predictive models estimating the effect of a treatment strategy. Here, two models were built that significantly predict the patient specific induced lymphocyte activity range (ILA-range) of various drugs based on supplemental patient information. To prove this concept, the machine-learning algorithms *ANN* and *CART* were used and the models derived were validated via training/validation set splitting and randomisation testing. Consequently, the objective was neither to optimise the models built nor to compare different machine learning algorithms. Nevertheless, in order to apply these models for treatment, the prediction accuracy of 63.0% (*CART*) and 61.1% (*ANN*) respectively is not sufficient and needs to be optimised. This can be

achieved, by adjusting the algorithms, increasing sample size and/or employing other algorithms.

In conclusion, this study not only proved the concept of computer simulations for the prediction of therapeutic regimens, but also proposed a general framework for analysing IET data. This framework includes crucial steps as data pre-processing for creating individual *immune efficiency profiles*, model building and evaluation of predictive models, and can be applied to all future studies analysing IET data.

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7 Appendix

7.1 IETDB Documentation (IET_Database.info)

The documentation file of the IETDB is accessible via the IET web interface and is also published on the IET-CD attached to this thesis. It summarises general information and attribute descriptions for the IET database and is reprinted below.

```
Citation Request:

     This Immuno Efficiency Test (IET) database was obtained from Davids
     Biotechnolgie and Dr. Hans-Albert Schöttler. If you publish results
     when using this database, then please include this information in
     your acknowledgements.


     1. Title: Immuno Efficiency Test (IET) Database (November, 2005)


     2. Sources:
        -- Dr. Michael Davids (Laboratory)
            Davids Biotechnologie
            Regensburg,
            Germany
        -- Donor: Dipl.-Ing. Hans-Albert Schöttler (Physician)
            Received by Dipl.-Ing. Hans-Albert Schöttler
        -- Date: 01 August 2005


     3. Past Usage:

     The dataset was used to determine patterns and build predictive
     models to estimate drug effects.


           1. Wolf, A.F-J. (2005). Storage and Analysis of Data Obtained
           from the Immune Efficiency Test (IET).


     4. Relevant Information:

     Samples arrive periodically as Dr. Davids and Dipl.-Ing. Schöttler
     report their clinical cases. The database therefore reflects this
     chronological grouping of the data. This grouping information
     appears immediately below.
```

Group 1:	1 patient	(0.95%)	(1999)
Group 2:	4 patients	(3.81%)	(2000)
Group 3:	8 patients	(7.62%)	(2001)
Group 4:	24 patients	(22.86%)	(2002)
Group 5:	12 patients	(11.43%)	(2003)
Group 6:	34 patients	(32.38%)	(2004)
Group 7:	22 patients	(20.95%)	(2005)

Total: 1241 patients

5. Number of Instances: 1241 (as of 01 August 2005)

6. Number of Attributes: 11

7. Attribute Information:

Attribute	Storage Type	Data Type	Value
Age	Real	Range	[22.5, 91.4]
Blastodermic layer	Integer	Set	0, 2, 4, 6, 8
Blood Group	Integer	Set	0, 2, 4, 6, 8
BMI	Real	Range	[16.7, 36.7]
Diagnosis ID	Integer	Set	1,…,24
Drug Group	Integer	Set	0, 1, 2, 3, 4, 5
Drug ID	Integer	Set	0,…,56
Lymphocyte No.	Real	Range	[0.31, 4.5]
Sex	Integer	Flag	2, 4
Status	Integer	Set	0, 2, 4

8. Diagnosis ID:

ID	Diagnosis	ID	Diagnosis
1	not known	13	Ovarian Ca.
2	Bronchial Ca.	14	Pancreas Ca.
3	Colon Ca.	15	Prostata Ca.
4	Control	16	Rectum Ca.
5	Esophagus Ca.	17	Renal Ca.
6	Glioma	18	Sigma Ca.
7	Intestinal Ca.	19	Squanous Epithelium Ca.
8	Leukemia	20	Stomach Cancer
9	Malignant Melanoma	21	Uterus Ca.
10	Mamma Ca.	22	Vaginal Ca.
11	Myosarcoma	23	Vesica Ca.
12	Nasopharynx Ca.	24	Immune Deficiency

9. Tumarmarker: CCR, LSA, CEA

CCR [Extension]:	Continuous Complete Remission (normal range<0,8)
LSA [mg/100ml]:	Lipid bound Sialic Acid (normal range<22mg/100ml)
CEA [ng/ml]:	Carcino Embryonic Antigen (normal range 0-3ng/ml)

```
10. Class distribution:
                  Benign:      18    (17.14%)
                  Malignant:   81    (77.14%)
                  Not Known:   6     (5.71%)



11. Drug Attribute: Aggregation


Some drugs can be merged based on their chemical structure or
groups. All currently known subgroups occurring within the database
are listed below:


          Group    Drug ID              Description

          Group 0  0                    Control
          Group 1  37, 41, 50, 56       Enzyme
          Group 2  13, 18, 22, 25, 26   Mistletoe
          Group 3  32, 33, 47, 48, 49   Thymus
          Group 4  30, 54, 55           Vitamin
          Group 5  All others           Others
```

7.2 Visual Programming in Clementine

Most data mining procedures were performed in Clementine by visual programming. All implementations in Clementine (called streams) are summarised in this section.

Data understanding – The stream implementation for data understanding is shown in Figure 23. First, data type settings for all attributes were made. This was done using a *Type* node. Storage types of the attributes were defined in the *InitialDataset* node.

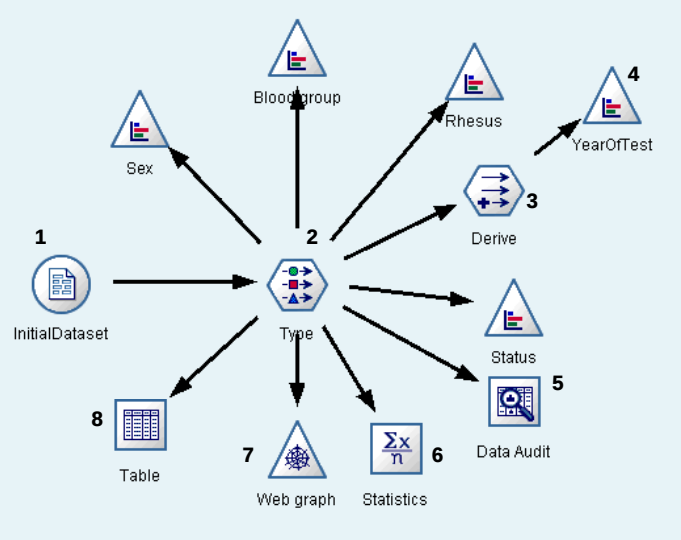

Figure 23 Clementine stream for *data understanding*. Visualisation of the initial dataset (1) aided to define data mining objectives. Clementine provides a set of tools to promote basic understanding of the dataset. To promote data understanding tables (8), distribution graphs (4), basic statistics tools (6) and general data audit illustrations (5) were used. The Type node (2) is used to define data types of the various attributes. A web graph (7) is used to determine interactions of the *Blood Group*, *Rhesus* and *Status* attributes. A *Derive* node (3) is used to generate the attribute *YearOfTest.*

For visualisation of the initial dataset, the Clementine tools *Table*, *Distribution graph*, *Statistics* and *Data Audit* were used. In order to create the new attribute *YearOfTest* a *Derive* node was used. Results of these operations, in particular the distribution of the attributes *Sex*, *Status* and *YearOfTest*, increase data understanding and are summarised in the documentation file, IET_Documentation.info. To identify interactions between the *Status*, *Blood Group* and *Rhesus* attributes, a *Web Graph* node was used.

Data Pre-processing – All dataset, data, attribute and formatting operations performed in Clementine and necessary to create individual immune efficiency profiles are displayed in Figure 24.

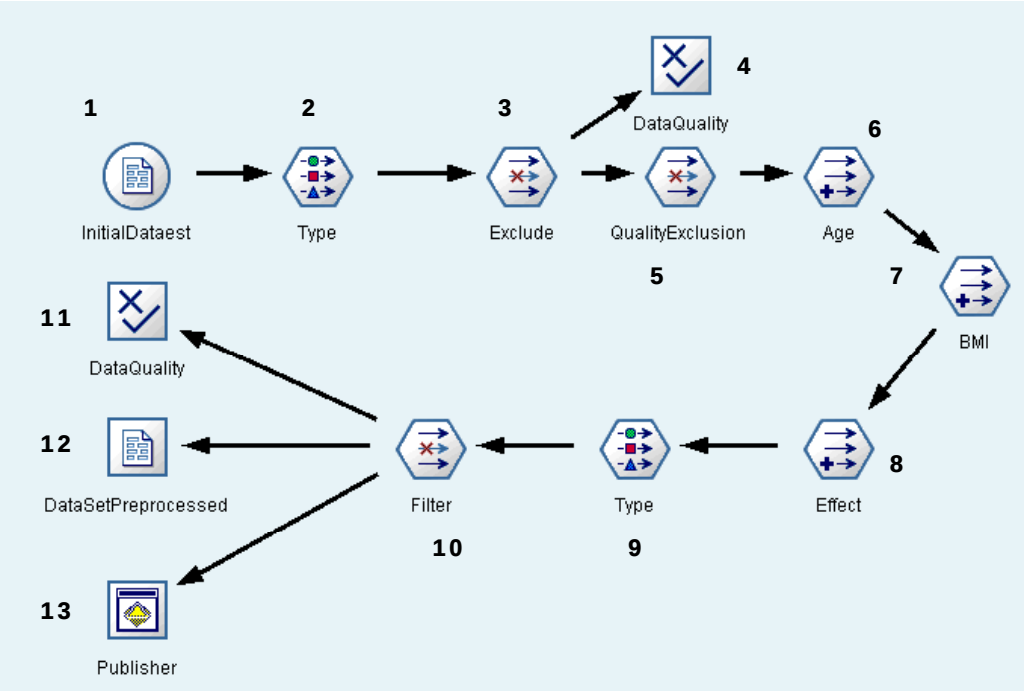

Figure 24 Clementine Stream for *Data Pre-processing*. The stream shows all operations required to prepare the dataset for modelling.

Before starting data pre-processing in Clementine, the dataset was transformed from a patient-wise into a drug-wise format by using the java application Transformer.java. Further, data types and missing values of the initial dataset (1) are set using a *Type* node (2). Irrelevant attributes (*Patient_ID*, *Test_ID* and *Postcode*) were excluded using a *Filter* node (3). Data quality analysis was performed using a *Quality* node (4) which automatically generates a Filter node (5) to exclude attributes of insufficient data quality. The derived *Age*, *BMI* and *Effect* attributes were included by using *Derive* nodes (6, 7, 8). A *Type* node (9) was used to set data types for these attributes. Thus, the original *Date of Birth*, *Date of Test*, *Height*, *Weight* and *Result* attributes were excluded using a *Filter* node (10). Data quality was analysed (11) once more, before accepting all data pre-processing operations by creating a new data file (12). Finally, the source code of the entire stream was published using a *Publisher* node (13). The source code of the stream can be found on the IET-CD.

Modelling – Before model building was started, the pre-processed dataset was split into a training and validation set. This was done using

the java application Randomiser.java. The Clementine stream of the modelling phase is illustrated in Figure 25.

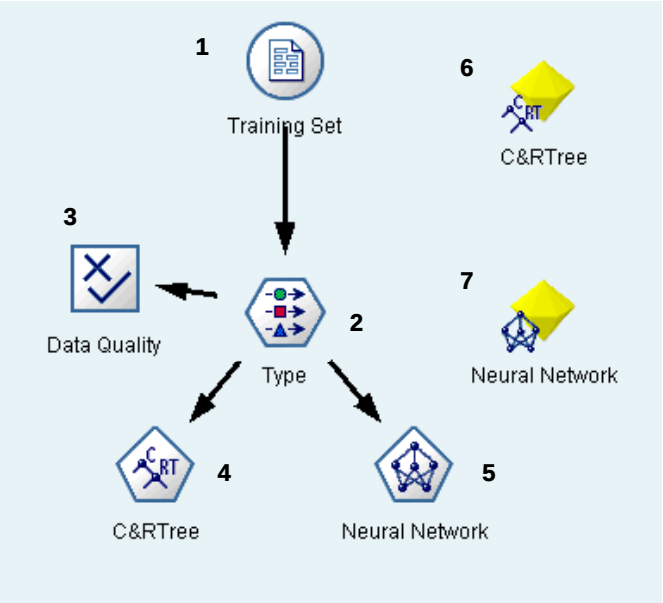

Figure 25 Clementine Stream Implementation for *Modelling*. In the *Modelling* phase, the two Clementine algorithms C&Rtree (4) and Neural Network (5) were used for model building.

In the modelling phase the machine learning algorithms *C&RTree* (4) and *Neural Network* (5) used the training set (1) for model building. Since dataset splitting was performed externally (Sampling methods provided by Clementine were not sufficient for our purposes; see Randomiser.java), data types and missing values needed to be defined again. This was done using a *Type* node (2). A *Quality* node (4) was used to analyse data quality before model building was started.

Evaluation – The evaluation phase (Figure 26) tested how well the two models (4, 5) performed on the validation set (1). This was done using *Quality* nodes (6, 7). The steps (2) and (3) were identical to the modelling phase. Finally, the stream source code was published for both models (8, 9). This includes all settings for model building as well.

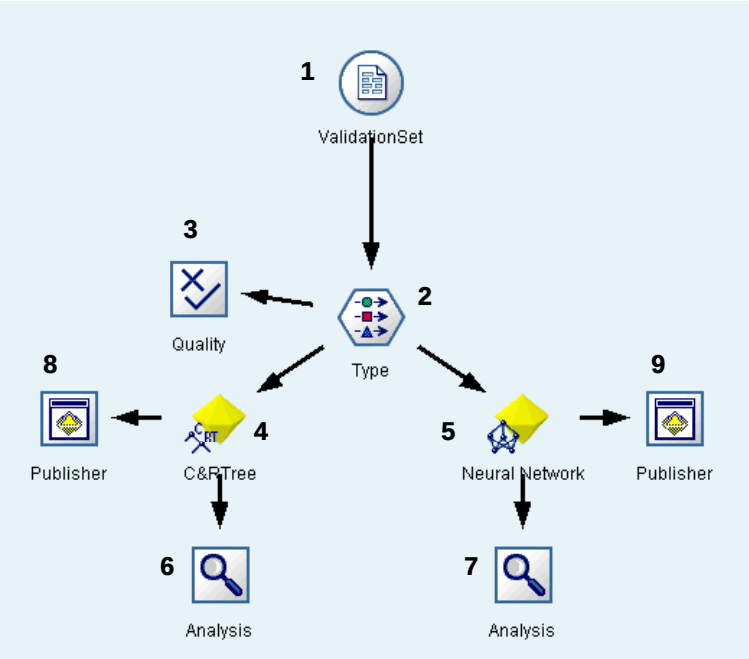

Figure 26 Clementine Stream Implementation for *Evaluation*. In the evaluation phase, quality of the models built in the modelling phase is assessed (6, 7).

7.3 Transformer.java

Transformer.java is a java application, implemented for transferring the initial IET dataset from a patient-wise into a drug-wise format. In doing so, Transformer.java replaces the columns *Drug 1* with *Drug 56* and the column *w/o Drug* with the columns *Drug ID* and *Result*. The column *Drug ID* holds the identifying numbers 0 to 56, where *0* represents the column *w/o Drug* and all other values (1 to 56) represent the former column names (*Drug 1* to *Drug 56*). The values of the original columns are transferred into the *Result* column. The source code of Transformer.java is published on the IET-CD attached to this thesis.

7.4 Randomiser.java

Randomiser.java randomly splits the original dataset into two separate, non-overlapping dataset and stores them with the suffices *_training* and *_validation* respectively. The splitting ratio is 30%/70%. This is implemented by setting the splitting ratio to 0.3 and generating random numbers (0 to 1) for each record. If the number generated is higher than 0.3, the respective record is allocated to the 70% split and vice

versa. The 30% split is used for validation, the 70% for training. The source code of Randomiser.java is published on the IET-CD.

7.5 RandomisationTester.java

As input file, RandomisationTester.java takes for example the IET validation set. The *number of permutation* and *number of bars* parameters for the resulting histogram can be altered in the source code. By default 10,000 permutations and 50 bars are used. RandomisationTester.java was implemented to perform randomisation testing not being provided in Clementine. Here, the class attribute *Effect* is randomly shuffled 10,000 times for the given dataset. These permutations are stored and compared to the original set. This results in a histogram representing the number of correct predictions by randomisation testing. The user gets a file with the suffix *_histogram* appended to the original file name, including histogram data, along with the standard deviation and the arithmetic mean. The source code is published on the IET-CD.

7.6 IET CD

The CD attached to this thesis is meant for specialists interested in the source code. Here, the source code of the following applications and implementations is published:

- **IET Database** – SQL statements for creating the database
- **IET Web Interface** – All html and php scripts developed for the interface
- **Clementine Visual Programming** – Code for all relevant streams including those for data pre-processing, modelling and evaluation
- **Transformer.java** – Source code written in java
- **Randomiser.java** – Source code written in java
- **RandomisationTester.java** – Source code written in java

8 Glossary

CGI

CGI is a standardised web technology for the communication of a client browser and a web server.

CRISP-DM

CRISP-DM is a industry proven standard methodology for data mining.

Data Type

A data type is a named category of data that is characterised by a set of values along with a description to denote those values and a collection of operations that interpret and manipulate the values.

Entity

An entity is something that has a distinct and separate existence. This does not necessarily imply a material existence.

Entity Type

A collection of entities characterised by common properties.

Java

Java is a object-oriented programming language.

JavaScript

JavaScript is a object-based scripting language used to include dynamic elements into web pages.

Kohonen

The kohonen algorithm implements the concept of self organising maps and assigns to unsupervised learning.

PERL

Perl is an interpreted procedural programming language developed by Larry Wall in 1987. Perl is synthesis of C, unix commands and other influences.

PHP

PHP is a programming language used to create websites. The syntax of php is loosely based on C and Perl.

Primary key

A primary key is a unique identifier (attribute) within a table, that is capable of distinguishing each row from all the other rows.

SQL

SQL is the most commonly used computer language to create, modify and retrieve data from a relational database management system.

Stream programming

Stream programming or visual programming provides an opportunity for the developer to build a program just by plugging pre-written pieces of logic together.

Table

A table is a set of elements with a horizontal dimension (rows) and a vertical dimension (columns).

9 List of Abbreviations

ACL	**A**ctual **C**lass **L**abel
AI	**A**rtificial **I**ntelligence
ANN	**A**rtificial **N**eural **N**etwork
CART	**C**lassification **A**nd **R**egression **T**ree
C&Rtree	**C**lassification **& R**egression **tree**
CGI	**C**ommon **G**ateway **I**nterface
CRISP-DM	**CR**oss **I**ndustry **S**tandard **P**rocess for **D**ata **M**ining
DBMS	**D**ata**B**ase **M**anagement **S**ystem
DMEM	**D**ulbecco/Vogt **M**odified **E**agle's Minimum Essential **M**edium
FCS	**F**etal **C**alf **S**erum
FISH	**F**luorescent **I**n **S**itu **H**ybridisation
IET	**I**mmune **E**fficiency **T**est
ILA	**I**nduced **L**ymphocyte **A**ctivity
KDD	**K**nowledge **D**iscovery in **D**atabases
LA	**L**ymphocyte **A**ctivity
NchIP	**N**ative **C**hromatin **I**mmuno **P**recipitation
PCL	**P**redicted **C**lass **L**abel
PCR	**P**olymerase **C**hain **R**eaction
PERL	**P**ractical **E**xtraction and **R**eport **L**anguage
PHP	**P**HP: **H**ypertext **P**reprocessor
PK	**P**rimary **K**ey
FK	**F**oreign **K**ey
q-PCR	**q** - **P**olymerase **C**hain **R**eaction
RCL	**R**andomised **C**lass **L**abel

rt-PCR	**r**eal **t**ime - **P**olymerase **C**hain **R**eaction
SQL	**S**tructured **Q**uery **L**anguage

10 List of Figures

11 List of Tables