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# Animal Models of Lymphoproliferative Disorders Focusing on Waldenström's Macroglobulinemia

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## 1. Introduction

Lymphoproliferative disorders (LPDs) represent a heterogeneous group of expanding, monoclonal or oligoclonal, lymphoid cells that occur in the setting of immune dysfunction. They are sometimes equated with "immunoproliferative disorders", but technically LPDs are a subset of immunoproliferative disorders, along with hypergammaglobulinemia and paraproteinemias. Several inherited gene mutations have been identified to cause lymphoproliferative disorders. Acquired and iatrogenic causes are also responsible for the appearance of these diseases.

The most common examples of LPDs are chronic lymphocytic leukemia, acute lymphoblastic leukemia, lymphomas/leukemias (including follicular lymphoma and hairy cell leukemia) and multiple myeloma, although less common LPDs such as post-transplant lymphoproliferative disorder, Waldenström's macroglobulinemia, Wiskott-Aldrich syndrome and Autoimmune LymphoProliferative Syndrome (ALPS) also belong to the same group of disorders.

A few basic current facts for the incidence rates, the prognosis and the treatment of the most common LPDs will be briefly mentioned in the beginning of this chapter. Following that, the recent advances in understanding the pathogenesis of these diseases coming from experimental animal studies will be reviewed in a greater detail. After all, understanding the mechanisms of neoplasia has always been a prerequisite for developing more effective treatments for cancer patients, and the sophisticated animal models available in our days have played a major role in enhancing this knowledge. We have developed an animal model for Waldenström's macroglobulinemia (WM), which is one of the less common LPDs. This will also be presented in detail as an example of the challenges met in developing an animal model that should emulate the human disease, or at least important aspects of it. By introducing core biopsies of WM patients into immunodeficient mice bearing human bone fragments, we established an animal model mimicking important aspects of the disease in humans.

## 2. Lymphoproliferative disorders

### 2.1 Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in North America and Europe; it is less frequent in Asia and Africa (Linnet et al., 2006). The reported age-adjusted incidence rate of CLL in the United States between 1975 and 2006 was 4.43 per 100,000 persons (Horner et al., 2009). However, because of its long asymptomatic period, the incidence of CLL is under-reported in cancer registries (Dores et al., 2007).

Despite this uncertainty, it is clear that the incidence of CLL rises dramatically with age and that it is more common in men than women (Dores et al., 2007; Redaelli et al., 2004). As the proportion of older people has increased with improved life expectancy in the Western world, the CLL burden has also increased. The American Cancer Society projected 15,490 new cases for 2009, a substantial increase from the 11,168 new cases reported in 2005 (U.S. Cancer Statistics Working group, 2009). The disease burden is also significant in the European Union, with an estimated 46,000 individuals in 2006 living with CLL 5 years post-diagnosis (Watson et al., 2008).

CLL is characterized by a variable clinical course (Rozman & Montserrat, 1995) with some patients having an aggressive malignancy and others a slow, nonprogressive disease and a virtually normal life expectancy. Ideally, a detailed diagnostic workup of a CLL case should include the identification of standardized and reliable prognostic factors. Predicting the outcome of CLL with a statistically significant level of success will provide the basis for individualized therapeutic approaches and patient-adjusted disease management policies. Indeed, several prognostic factors, including serum (Hallek et al., 1999) and cytogenetic alteration markers (Dohner et al., 2000), have been used to assist individual CLL patient prognosis.

### 2.2 Acute lymphoblastic leukemia

There are two types of acute leukemia: acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL); (Ashfaq et al., 2010). Acute myelogenous leukemia (AML) is a clonal, malignant disease of hematopoietic tissue that is characterized by the proliferation of abnormal (leukemic) blast cells, principally in the marrow, and by impaired production of normal blood cells (Lichtman & Liesveld, 2001). It is the most common acute leukemia affecting adults, and its incidence increases with age. AML accounts for nearly one-third of all new cases of leukemia (Ashfaq et al., 2010).

Acute lymphoblastic leukemia is a neoplastic disease that results from somatic mutation in a single lymphoid progenitor cell at one of several discrete stages of development. The immunophenotype of the leukemic cells at diagnosis reflects the level of differentiation achieved by the dominant clone (Pui, 2001). At diagnosis the leukemic cells not only have replaced normal marrow cells but have disseminated to various extramedullary sites. Studies suggest that the activation of telomerase in leukemic cells contribute to their growth advantage and to disease progression (Ohyashiki et al., 1997; Shay et al., 1996).

ALL represents about 12 percent of all leukemias diagnosed in the US, and 60 percent of all cases occur in persons younger than 20 years (SEER, 1998). ALL is the most common malignancy diagnosed in patients under the age of 15 years, accounting for one-fourth of all

cancers and 76 percent of all leukemias in this age group (Gurney et al., 1996). Data from UK showed that there were 691 new cases of ALL and 255 deaths from ALL in 2006 (Cancer Research U.K., 2010). Each year, around 3,250 children are diagnosed with leukemia, of which about 2,400 are ALL cases (Smith et al., 2000). In the USA, survival rate for children with ALL has improved markedly since the early 1970s and is now approximately 80%, but incidence rates have not decreased and have, in fact, increased by 0.8% annually from 1975 to 2007 (SEER, 2010). Worldwide, according to the World Health Organization (WHO), there were 33,142 deaths from leukemia among children under age 15 in 2004, and childhood (<15 years) leukemia caused 1,228,075 disability adjusted life years (WHO, 2010).

Identifying risk factors for childhood leukemia is an important step in the reduction of the overall burden of childhood diseases. Though it has been studied intensively, the etiology of childhood leukemia is not well established. A two-hit model was proposed by Greaves in which prenatal chromosome alterations and postnatal genetic alterations are necessary for childhood leukemia development (Greaves, 2002). Genetic susceptibility and environmental factors play potential roles in this process (Eden, 2010). Ionizing radiation has been significantly linked to childhood leukemia (Bailey et al., 2010). Evidence for an association with benzene exposure or with parental smoking and alcohol consumption is less convincing (Liu et al., 2011).

Treatment for the majority of ALL subtypes consists of three phases: induction, intensification (consolidation) therapy, and continuation (maintenance) treatment. Although two-thirds of childhood cases are curable with only 12 months of treatment, the vast majority of patients undergo therapy for two years or more (Pui & Evans, 2006). Across medical institutions, chemotherapeutic agents used vary in type and amount, with the most common being methotrexate (MTX), cytosine arabinoside (cytarabine), anthracyclines (such as doxorubicin), asparaginase, mercaptopurine, vincristine, and corticosteroids, presented alone or in combination (Pui & Evans, 2006). Leukemic cells are transported by the circulatory system to nearly every organ system, including the Central Nervous System (CNS). The most common form of CNS prophylaxis was cranial irradiation, or cranial radiation therapy (CRT), which has largely been replaced by intrathecal (IT) and systemic chemotherapy. This change has been made in an effort to eliminate radiation-specific damage to the CNS (Stehbens et al., 1991). Recent regimens have tested whether CRT can be eliminated completely from standard treatment. To date, this has been successful, although alterations in long-term outcome are just beginning to unfold (Pui et al., 2009). Efforts like this are being made to eliminate the possible complications of any form of ALL treatment.

### **2.3 Lymphomas/leukemias**

Despite remarkable advances in diagnosis and treatment, lymphoma continues to rank as a leading cause of cancer-related mortality. Recent cancer statistics for the United States project non-Hodgkin lymphoma (NHL) to be the sixth most commonly diagnosed cancer in 2010 in both men and women, and the eighth and sixth leading cause of cancer-related death in men and women, respectively (Jemal et al., 2010). Based on data from national cancer registries, 65,540 new cases of NHL and 20,210 deaths from NHL are estimated to occur in 2010. In contrast, Hodgkin lymphoma (HL) is less common (8,490 estimated new cases in 2010) and is associated with fewer deaths (1,320 estimated deaths in 2010) (Jemal et al., 2010). In the European Union, reported NHL estimates for the year 2006 were even

higher, with 72,800 new cases and 33,000 deaths (Ferlay et al., 2007). In the US, on January 1, 2008, there were approximately 167,000 HL survivors and approximately 454,000 NHL survivors (Howlader et al., 2011). In the Nordic European Countries (NEC: Denmark, Faroe Islands, Finland, Iceland, Norway, Sweden), there were approximately 10,500 HL survivors and approximately 31,500 NHL survivors at the end of 2007 (Engholm et al., 2011). Although there are similarities between these subtypes of lymphoma, the incidence and age of onset are quite different.

Onset of the disease occurs most frequently between the ages of 20 and 35 years. Between 35 and 50 years, it occurs less often especially in females, but from the age of 50 onward there is again a rise in incidence with age (Howlader et al., 2011). The disease occurs predominantly in individuals aged over 45 years and the lifetime prevalence of NHL is one in 50 (Howlader et al., 2011). Due to chemotherapy, radiotherapy and stem cell transplantation, the survival of these patients has improved substantially in the seventies and eighties, but has nowadays leveled off. In effect, most trials focus on maintaining the high level of cure, while reducing the long-term effects of treatment. To date, more than 80% of patients diagnosed with HL are expected to live free of disease for 5 years or more after diagnosis (National Cancer Institute, 2009) The overall 5-year survival rate for all types of NHL (1999–2005) is 50–60%. The statistics vary depending on the cell type, stage of disease at diagnosis, treatment and age of the patient (National Cancer Institute, 2009).

Indolent Non-Hodgkin's lymphoma (NHL) represents a group of incurable slow growing lymphomas that are highly responsive to initial therapy but relapse with less responsive disease (Ardeshna et al., 2003; Horning, 1993; Johnson et al., 1995; Montoto et al., 2002). The landscape for treatment of indolent NHL has dramatically changed with the introduction of rituximab (Rituxan, Genetech, San Francisco, CA). Its greatest impact has been in follicular lymphoma (FL), which constitutes approximately 70% of indolent lymphomas and up to 25% of all cases of NHL (Marcus et al., 2005; The Non-Hodgkin's Lymphoma Classification Project, 1997). Although there are no defined first line therapies for indolent NHL, rituximab has become a standard component in treatment of Follicular Lymphoma (FL) (Friedberg et al., 2009). While indolent lymphoma remains an incurable disease, recent data from the Surveillance Epidemiology and End Results (SEER) database and retrospective analysis of clinical trials in indolent NHL suggest an improved overall survival with the use of rituximab (Fisher et al., 2005; Liu et al., 2006; Pulte et al., 2008). It is hoped that overall survival can be further improved with the use of extended rituximab dosing schedules.

#### **2.4. Multiple Myeloma**

Multiple Myeloma (MM) is a B cell malignancy characterized by the presence of bone marrow infiltration by clonal plasma cells that generally secrete a monoclonal component in the serum or urine (Kyle & Rajkumar, 2004). It is the second most frequent hematological malignancy, after non Hodgkin's lymphomas, and accounts approximately for a 10% of all hematological tumors and 1% of all cancers (Petrelli et al., 2009). MM is associated with a constellation of disease manifestations, including osteolytic lesions due to disrupted bone metabolism, anemia and immunosuppression due to loss of normal hematopoietic stem cell function, and end-organ damage due to monoclonal immunoglobulin secretion (Barlogie et al., 2001). The presence of somatic hypermutations of the immunoglobulin variable region genes in myeloma plasma cells suggests that malignant transformation occurs in a B cell that

has traversed the germinal centers of lymph nodes. However, the hypoproliferative nature of myeloma has led to the hypothesis that the bulk of the tumor arises from a transformed B cell with the capacity for both self-renewal and production of terminally differentiated progeny (Billadeau et al., 1993; Corradini et al., 1993; Szczepek et al., 1998).

The clinical course of patients requiring therapy for myeloma varies markedly. Even with tandem autotransplants yielding complete remission (CR) rates in excess of 60%. Survival ranges from a few months to greater than 15 years. The extended time (almost 2 years) for those patients to achieve CR, and the even longer time to achieve magnetic resonance imaging (MRI)-CR, strongly suggests enormous tumor cell population heterogeneity in terms of drug responsiveness/resistance (Harousseau et al., 2004).

Differential expression of traditional prognostic factors, such as  $\beta$ 2-microglobulin ( $\beta$ 2M), albumin, and C-reactive protein (CRP), are thought to be responsible for only a 15%–20% of the outcome heterogeneity of MM. Abnormal metaphase karyotypes, present in one-third of newly diagnosed patients and reflecting stroma independence, have been consistently associated with a rapidly fatal outcome, and fewer than 10% of patients with these abnormalities survive > 5 years (Harousseau et al., 2004).

Advances in molecular cytogenetics have identified primary translocations involving the immunoglobulin heavy chain locus at 14q32 in 40% of patients (Kuehl & Bergsagel; 2002). According to a consensus report of a Paris workshop on myeloma genetics, hyperdiploid and t(11;14)(q13,q32)-positive myeloma are associated with a good prognosis, whereas non-hyperdiploidy, often associated with translocations other than t(11;14) and chromosome 13 deletion, imparts a strikingly dismal prognosis (Fonseca et al., 2004).

## 2.5 Other lymphoproliferative disorders

Along with Waldenström's macroglobulinemia, there are other LPDs, which are less frequently observed. From this group we will describe in more details the lymphoproliferative disorders detected after bone marrow and organ transplantation. Following this treatment and among patients infected with AIDS, LPDs are believed to result from uncontrolled proliferation of Epstein-Barr virus (EBV)-transformed B-lymphocytes in the setting of immune dysfunction (Cohen, 1991; Deeg & Socié, 1998; Goedert et al., 1998; Hoover, 1992; Kinlen, 1996; Newell et al., 1996; Opelz & Henderson, 1993; Swinnen et al., 1990). Allogeneic bone marrow transplantation, an effective treatment for leukemia and other disorders, produces profound immune deficiency in the early period after transplantation. Post-transplant lymphoproliferative disorders (PTLD) are an uncommon, but frequently fatal, complication of this defective immune function (Bhatia et al., 1996; Deeg & Socié, 1998; Witherspoon et al., 1989). PTLD typically develop in the first 6 months post-transplantation as clinically aggressive lymphomas of donor origin; most are related to EBV (Deeg & Socié, 1998; Orazi et al., 1997; O'Reilly et al., 1996).

Post-transplant lymphoproliferative disorders are more common if donor and recipient are HLA-mismatched or if T-cell depletion is used for graft-versus-host disease (GVHD) prophylaxis (Curtis et al., 1999). The clinical diagnosis of PTLD may be difficult because it is a spectrum of heterogeneous histologic and clinical entities. It may present as an infectious mononucleosis-like illness, with fatigue and lymphadenopathy, or as a febrile illness with leukopenia. Almost all organs may be affected by disease. Because of the progressive nature

of PTLTD, the key to management may be early or even preemptive treatment with either anti-B-cell monoclonal antibodies (Carpenter et al., 2002; Kuehnle et al., 2000; van Esser et al., 2002) or donor-derived EBV-specific cytotoxic T lymphocytes (CTLs) (Gustafsson et al., 2000; Heslop et al., 1996; Rooney et al., 1998).

### 3. Animal models of lymphoproliferative disorders

The bulk of LPD-related literature describes the different clinical manifestations encountered and evaluates treatment protocols. Fewer studies focus on dissecting the pathogenesis of LPD by developing animal models. Recent basic research is based on the development of genetically engineered mice or the use of immunodeficient mice as tumor transplant models. A few animal models other than mice have also been reported (miniature swine, zebrafish).

#### 3.1 Chronic lymphocytic leukemia

Chronic lymphocytic leukemia cells require complex microenvironmental and immunologic interactions to survive and proliferate. Such interactions might be best studied in animal models; however, this needs extensive verification. Hofbauer et al. (2011) therefore investigated the composition of the T-cell compartment in the E $\mu$ -*TCL1* transgenic mouse, currently the most widely used murine model for CLL. *TCL1* is a bona fide oncogene, developing a transgenic mouse model where ectopic expression driven by the *lck* promoter in the T cell compartment results in the development of mature T cell leukemias after a long latency period, in a pattern closely resembling human mature T cell leukemia (Virgilio et al., 1998). Immunophenotyping and transplant approaches were used to define T-cell subsets at various stages of CLL. Analogous to human CLL, they observed a skewing of T-cell subsets from naive to antigen-experienced memory T cells that was more pronounced in lymph nodes than in blood. Transplantation of CLL into non-transgenic recipients was feasible without immunosuppression in a pure C57BL/6 background and resulted in the prominent skewing of the T cells of the recipient mice. Both in spontaneously developed CLL and in the transplantation setting, a loss in T-cell receptor diversity was observed, with a relevant number of clonal T-cell populations arising. This suggests that antigen-dependent differentiation toward the T memory pool is initiated by murine CLL cells. In summary, this research team characterized the T-cell phenotypes in the *TCL1* transgenic mouse model and suggested a CLL-dependent antigen-driven skewing of T cells in these mice, making this model valuable for the research of the disease's pathogenesis, since murine CLL cells react similarly with human CLL cells.

The same transgenic mouse (E $\mu$ -*TCL1* transgenic mouse,) was also used by Suljagic et al. (2010). They have investigated whether inhibition of BCR (antigen-dependent B-cell receptor) signaling with the selective Syk inhibitor fostamatinib disodium (R788) had an affect on the growth of the leukemias that develop in the E $\mu$ -*TCL1* transgenic mouse model of CLL. Similarly to human CLL, these leukemias express stereotyped BCRs that react with autoantigens exposed on the surface of senescent or apoptotic cells, suggesting that they are antigen driven. They showed that R788 effectively inhibits BCR signaling *in vivo*, resulting in reduced proliferation and survival of the malignant B cells and significantly prolonged survival of the treated animals. The growth-inhibitory effect of R788 occurs despite the relatively modest cytotoxic effect *in vitro* and is independent of basal Syk activity,

suggesting that R788 functions primarily by inhibiting antigen-dependent BCR signals. Importantly, the effect of R788 was found to be selective for the malignant clones, as no disturbance in the production of normal B lymphocytes was observed. Collectively, these data provide further rationale for clinical trials with R788 in CLL and establish the BCR-signaling pathway as an important therapeutic target in this disease.

In another approach, over-expression of human *TCL1*, leads to the development of mature CD19<sup>+</sup>/CD5<sup>+</sup>/IgM<sup>+</sup> clonal leukemia with a murine disease phenotype similar to the human CLL. Herein, Chen et al. (2009) review their recent study using this *TCL1*-driven mouse model for CLL and corresponding human CLL samples in a cross-species epigenomics approach to address the timing and relevance of epigenetic events occurring during leukemogenesis. They demonstrated that the mouse model recapitulates the epigenetic events that have been reported for human CLL, affirming the power and validity of this mouse model to study early epigenetic events in cancer progression. Epigenetic alterations are detected as early as three months after birth, far before disease manifests at about 11 months of age. These mice undergo NFκ-B repressor complex-mediated inactivation of the transcription factor Foxd3, whose targets become aberrantly methylated and silenced in both mouse and human CLL. Overall, their data suggest the accumulated epigenetic alterations during CLL pathogenesis as a consequence of gene silencing through *TCL1* and NFκ-B repressor complex, suggesting the relevance for NFκ-B as a therapeutic target in CLL.

Another transgenic mouse model for CLL was generated by Santanam et al. (2010). They found that miR-29a is up-regulated in indolent human B-CLL as compared with aggressive B-CLL and normal CD19(+) B cells. To study the role of miR-29 in B-CLL, they generated Eμ-miR-29 transgenic mice overexpressing miR-29 in mouse B cells. Flow cytometric analysis revealed a markedly expanded CD5(+) population in the spleen of these mice starting at 2 months of age, with 85% (34/40) of miR-29 transgenic mice exhibiting expanded CD5(+) B-cell populations, a characteristic of B-CLL. On average, 50% of B cells in these transgenic mice were CD5 positive. At 2 years of age the mice showed significantly enlarged spleens and an increase in the CD5(+) B-cell population to approximately 100%. Of 20 Eμ-miR-29 transgenic mice followed to 24-26 mo of age, 4 (20%) developed frank leukemia and died of the disease. These results suggest that the dysregulation of miR-29 can contribute to the pathogenesis of indolent B-CLL, giving another opportunity to clarify all of its aspects.

The engraftment of cell lines into appropriate mice and/or the injection of fresh cells derived from patients have been used for the development of animal models in different diseases. Here, we describe one animal model where the researchers have developed a novel transplantable xenograft murine model of CLL by engrafting the CLL cell line MEC1 into Rag2(-/-)gamma(c)(-/-)mice. These mice lack B, T, and natural killer (NK) cells, and, in contrast to nude mice that retain NK cells, appear to be optimal recipient for MEC1 cells, which were successfully transplanted through either subcutaneous or intravenous routes. The result is a novel *in vivo* model that has systemic involvement, develops very rapidly, allows the measurement of tumor burden, and has 100% engraftment efficiency. This model closely resembles aggressive human CLL and could be very useful for evaluating both the biologic basis of CLL growth and dissemination as well as the efficacy of new therapeutic agents (Bertilaccio et al., 2010).

Another model has been developed by Aydin et al. (2011), and is exploring the role of CD38 and functionally associated molecular risk factors in a recently described CLL nonobese

diabetic/severe combined immunodeficient xenograft model. Intravenous injection of peripheral blood mononuclear cells from 73 patients with CLL into 244 mice resulted in robust engraftment of leukemic cells into the murine spleens detected 4 weeks after transplantation. Leukemic cell engraftment correlated significantly ( $P < 0.05$ ) with markers reflecting disease activity, e.g., Binet stage and lymphocyte doubling time, and the expression of molecular risk factors including CD38, CD49d, ZAP-70, and IgVH mutational status. Increased engraftment levels of CD38<sup>+</sup> as compared to CD38<sup>-</sup> CLL cells could be attributed, in part, to leukemic cell proliferation as evidenced by simultaneous immunostaining of murine spleen sections for Ki-67 and CD20. In short-term (24 h) homing assays, CD38<sup>+</sup> CLL cells migrated more efficiently to the bone marrow of the recipient animals than their CD38<sup>-</sup> counterparts. Finally, the expression of CD38 by the leukemic cells was found to be dynamic in that it was regulated not only by elements of the murine microenvironment but also by co-engrafting non-malignant human T cells. This model could be useful for evaluating the biological basis of CLL growth in the context of the hematopoietic microenvironment as well as for preclinical testing of novel compounds.

Last, but not least we describe the New Zealand Black (NZB) mouse model for CLL. Is a (*de novo*) mouse model of spontaneous CLL (Phillips et al., 1992), in contrast to all other models, which are induced by the expression of exogenous genes (Scaglione et al., 2007). Similar to a subset of human patients who progress from monoclonal B lymphocytosis (MBL) to CLL, NZB mice develop an age-associated progression to CLL. The murine disease is linked to a genetic abnormality in microRNA *mir-15a/16-1* locus, resulting in decreased mature miR-15a/16 (Salerno et al., 2010).

Similar to CLL, the disease in NZB mice is also an age-associated malignant expansion of poly-reactive CD5<sup>+</sup> B-1 clones (Caligaris-Cappio & Ghia, 2007; Scaglione et al., 2007). The majority of B-1 clones are IgM<sup>+</sup>, B220 (CD45R)<sup>dim</sup> and CD5<sup>dim</sup>, increase with age, and often possess chromosomal abnormalities (Dang et al., 1996). NZB also seem to demonstrate an MBL-like stage at an early age, characterized by multiple clones, as seen in MBL cases reported by Lanasa et al. (2010). High levels of IL-10 are also correlated with the development of these malignant B-1 cells (Ramachandra et al., 1996). This MBL-like state in NZB precedes CLL, and although it exhibits similar manifestations to human MBL, NZB disease always progress to CLL, in contrast to humans who can have an indefinite state of indolent MBL disease (Lanasa et al., 2010). The NZB has also been studied as a model for autoimmunity (Theofilopoulos, 1996). Similar to the autoreactivity associated with CLL autoantibodies (Ghia et al., 2002), the NZB mouse develops a mild autoimmune reaction associated with B cell hyperactivity, resulting in autoimmune hemolytic anemia and antinuclear antibodies (Scaglione et al., 2007).

The diversity of existing animal models of CLL leads to multiple options for treatment approaches, which is the end-point of this research.

### 3.2 Acute lymphoblastic leukemia

The non-obese diabetic/severe combined immunodeficient (NOD/SCID) xenograft mouse model is currently one of the most successful models for studying haematological malignancies such as acute lymphoblastic leukaemia (ALL) (Macor et al., 2008). In this typical tumor transplant model patient bone marrow leukemia cells are directly

transplanted into the recipient NOD/SCID mice (Lock et al., 2002). The kinetics of engraftment reflect the human disease, leading to bone marrow (BM) infiltration, followed by migration to the spleen, peripheral blood and other haematopoietic organs (Lock et al., 2002; Kamel-Reid et al., 1989; Nijmeijer et al., 2001). However, for this model to be effective for studying engraftment and therapy responses at the whole genome level, careful molecular characterization is essential.

In the ALL NOD/SCID xenograft model, Samuels et al. (2010) have combined all existing xenograft models and sought to validate species-specific gene expression. Using the human Affymetrix whole transcript platform they analyzed transcriptional profiles from engrafted tissues (e.g. bone marrow, spleen and/or peripheral blood) without prior cell separation of mouse cells and acquired highly reproducible profiles in xenografts from individual mice. The model was further tested with experimental mixtures of human and mouse cells, demonstrating that the presence of mouse cells does not significantly distort expression profiles when xenografts contain 90% or more human cells. In addition, they presented a novel *in silico* and experimental masking approach to identify probes and transcript clusters susceptible to cross-species hybridization. Hence, they demonstrated that species-specific transcriptional profiles can be obtained from xenografts when high levels of engraftment are achieved or with the application of transcript cluster masks. Importantly, this masking approach can be applied and adapted to other xenograft models where human tissue infiltration is lower. This model provides a powerful platform for identifying genes and pathways associated with ALL disease progression and response to therapy *in vivo*.

A genetically defined mouse retroviral transduction/bone marrow transplantation model was used by Medyouf et al. (2010) to investigate the possibility for NOTCH1 to act as a therapeutic target. This is based on the assumption that NOTCH1 is activated by mutation in more than 50% of human T-cell acute lymphoblastic leukemias (T-ALLs) and inhibition of Notch signaling causes cell-cycle/growth arrest. The tumor suppressor phosphatase and tensin homolog (PTEN) is also mutated or lost in up to 20% of cases. It was recently observed among human T-ALL cell lines that PTEN loss correlated with resistance to Notch inhibition, raising concern that patients with PTEN-negative disease may fail Notch inhibitor therapy. They observed primary murine leukemias to remain dependent on NOTCH1 signaling despite *Pten* loss, with or without additional deletion of *p16(Ink4a)/p19(Arf)*. They also examined 13 primary human T-ALL samples obtained at diagnosis and found no correlation between PTEN status and resistance to Notch inhibition. Furthermore, they noted that *Pten* loss accelerated disease onset and produced multiclonal tumors, suggesting NOTCH1 activation and *Pten* loss may collaborate in leukemia induction. Thus, in contrast to previous findings with established cell lines, these results indicate that PTEN loss does not relieve primary T-ALL cells of their "addiction" to Notch signaling. They concluded that refractory/relapsed tumors that have undergone chemotherapy-induced mutation and/or selection may behave differently, but presumably will harbor many other genetic alterations besides PTEN loss. This conclusion, along others of the same research team provide new insight on the therapeutic management of ALL.

Introduction of cells into syngeneic mice is also useful tool for the investigation of ALL and its therapeutic approach. Cultured *p185(BCR-ABL)*-expressing (*p185+*) *Arf* (-/-) pre-B cells injected into healthy syngeneic mice induces aggressive acute lymphoblastic leukemia (ALL) that genetically and phenotypically mimics the human disease (Boulos et al., 2011).

They adapted the Philadelphia chromosome-positive (Ph(+)) ALL animal model for *in vivo* luminescent imaging to investigate disease progression, targeted therapeutic response, and ALL relapse in living mice. Mice bearing high leukemic burdens (simulating human Ph(+)) ALL at diagnosis) entered remission on maximally intensive, twice-daily dasatinib therapy, but invariably relapsed with disseminated and/or central nervous system disease. Their research concluded that although non-tumor-cell-autonomous mechanisms can prevent full eradication of dasatinib-refractory ALL in this clinically relevant model, the emergence of resistance to BCR-ABL kinase inhibitors can be effectively circumvented by the addition of "conventional" chemotherapeutic agents with alternate antileukemic mechanisms of action. Thus, preclinical trials using multiple agents underscore the potential value of this murine Ph<sup>+</sup> ALL model for efficiently and cheaply piloting combination therapies and for elucidating mechanisms of drug resistance, information that is much more difficult to extract from complex human clinical trials.

Other researchers have combined ALL models with exogenous factors, to test their influence in disease. For example, Yun et al. (2010) developed animal models of obesity and leukemia to test whether obesity could directly accelerate acute lymphoblastic leukemia (ALL) using BCR/ABL transgenic and AKR/J mice weaned onto a high-fat diet. Mice were observed until development of progressive ALL. Although obese and control BCR/ABL mice had similar median survival, older obese mice had accelerated ALL onset, implying a time-dependent effect of obesity on ALL. Obese AKR mice developed ALL significantly earlier than controls. The effect of obesity was not explained by WBC count, thymus/spleen weight, or ALL phenotype. However, obese AKR mice had higher leptin, insulin, and interleukin-6 levels than controls, and these obesity-related hormones all have potential roles in leukemia pathogenesis. In conclusion, obesity directly accelerates presentation of ALL, likely by increasing the risk of an early event in leukemogenesis. This is the first study to show that obesity can directly accelerate the progression of ALL. Thus, the observed associations between obesity and leukemia incidence are likely to be directly related to biological effects of obesity.

Smith et al. (2010) used another animal, zebrafish, where malignant cells can be transplanted into sibling animals without the need for immune suppression. Using cell-transplant zebrafish (Langenau et al., 2003) showed that self-renewing cells are abundant in T-ALL and comprise 0.1% to 15.9% of the T-ALL mass. Large-scale single-cell transplantation experiments established that T-ALLs can be initiated from a single cell and that leukemias exhibit wide differences in tumor-initiating potential. T-ALLs can also be introduced into clonal-outcrossed animals, and T-ALLs arising in mixed genetic backgrounds can be transplanted into clonal recipients without the need for major histocompatibility complex matching. Finally, high-throughput imaging methods are described that allow large numbers of fluorescent transgenic animals to be imaged simultaneously, facilitating the rapid screening of engrafted animals. These experiments show that large numbers of zebrafish can be experimentally assessed by cell transplantation and establish new high-throughput methods to functionally interrogate gene pathways involved in cancer self-renewal.

### 3.3 Lymphomas/leukemias

There are numerous studies utilizing mouse models to study different lymphomas/leukemias. Here, only a few representative are mentioned, since the thorough report of these

models is beyond the scope of this chapter. As with other LPDs, the use of knock-out and/or transgenic mice has been substantial to study lymphomas and leukemias.

The first animal model we describe studies the pathogenesis of multiple hematopoietic malignancies simultaneously. The researchers (Zhang et al., 2011) have generated inducible Pten/Myc double-knockout mice (Pten(-/-)/Myc(-/-)). By comparing the hematopoietic phenotypes of these double-knockout mice with those of Pten(-/-) mice, they found that both sets of animals developed myelo- and lympho-proliferative disorders. Their study suggests that the deregulation of phosphoinositide 3-kinase/Akt signaling in Pten(-/-) hematopoietic cells protects these cells from apoptotic cell death, resulting in chronic proliferative disorders. Since, none of the compound-mutant mice developed acute leukemia or lymphoma, it is concluded that Myc is absolutely required for the development of acute hematopoietic malignancies.

Other researchers (Mukherjee et al., 2011) managed to develop spontaneous T- and B-cell lymphomas, and leukemia in mice: Homozygous deletion of ESPL1 gene that encodes Separase protein (Cohesin protease Separase plays a key role in faithful segregation of sister chromatids by cleaving the cohesin complex at the metaphase to anaphase transition) results in embryonic lethality in mice and Separase overexpression lead to aneuploidy and tumorigenesis. By examining the ESPL1 heterozygosity using a hypomorphic mouse model that has reduced germline Separase activity, they reported that while ESPL1 mutant (ESPL1(+ /hyp)) mice have a normal phenotype, in the absence of p53, mice develop spontaneous T- and B-cell lymphomas, and leukemia with a significantly shortened latency as compared to p53 null mice. Their results indicate that reduced levels of Separase act synergistically with loss of p53 in the initiation and progression of B- and T- cell lymphomas, which is aided by increased chromosomal missegregation and accumulation of genomic instability. ESPL1(+ /hyp), p53(-/-) mice provide a new animal model for mechanistic study of aggressive lymphoma and also for preclinical evaluation of new agents for its therapy.

An interesting murine model of diffuse large B-cell lymphoma (DLBCL) described by Yu et al. (2011), uses human DLBCL cell line LY8, to investigate its characteristics of growth pathogenesis and the effect of treatment protocols. LY8 cells were injected subcutaneously into the right flank of nude mice. Harvested tumor tissues were cut into small pieces of 1.5 mm × 1.5 mm × 1.5 mm and implanted subcutaneously into nude mice. Tumor growth was monitored and the histologic characteristics were documented. Expression of LCA, CD20, CD79 $\alpha$ , Ki-67, CD3, CD45RO, bcl-6, MUM-1, CD10 and bcl-2 were examined by using immunohistochemistry. IgH clonal rearrangement and status of three microsatellite loci (D14S68, D18S69, D20S199) in the xenografted tumor samples and the parental cell line LY8 were detected using PCR amplification followed by PAGE. The subcutaneous xenograft DLBCL model was successfully established by using cell line LY8, and a stable growth was achieved up to the 9th generation. The tumor in each generation showed similar growth characteristics and the rate of subcutaneous tumor formation was 91.9% (114/124). The tumor growth was observed from the 2nd week after morphological characteristics with those of human DLBCL, and expressed LCA, CD20, CD79 $\alpha$ , bcl-6, MUM-1, CD10 and bcl-2. The tumor of xenograft mice and cell line LY8 showed identical IgH rearrangement and microsatellite length. This mouse model recapitulates many features of human DLBCL with high stability and repeatability. Therefore, it provides an ideal animal model for in vivo studies of the biological characteristics and treatment of DLBCL.

Gaurnier-Hausser et al. (2011) set out to determine whether dogs with spontaneous DLBCL (diffuse large B-cell lymphoma) have comparative aberrant constitutive NF- $\kappa$ B activity and to determine the therapeutic relevance of NF- $\kappa$ B inhibition in dogs with relapsed, resistant DLBCL. Constitutive canonical NF- $\kappa$ B activity and increased NF- $\kappa$ B target gene expression were detected in primary DLBCL tissue. NF- $\kappa$ B essential modulator (NEMO)-binding domain (NBD) peptide inhibited this activity and induced apoptosis of primary malignant B cells *in vitro*. Intratumoral injections of NBD peptide to dogs with relapsed DLBCL inhibited NF- $\kappa$ B target gene expression and reduced tumor burden. This work shows that dogs with spontaneous DLBCL share therapeutic relevance of NF- $\kappa$ B inhibition in the treatment of ABC-DLBCL. These results have important translational relevance for ABC-DLBCL treatment in human patients, and dogs with spontaneous DLBCL may represent a clinically relevant, spontaneous, large animal model for human ABC-DLBCL.

Other findings suggest that increasing levels of human-derived IgG in peripheral blood from hu-PBL/SCID mice could be used to monitor EBV-related human B-cell lymphoma development in experimental animals (Tang et al., 2011). Epstein-Barr virus (EBV) has a close association with various types of human lymphomas. Tang et al. (2011) aimed to evaluate the association between human IgG concentration and EBV-associated lymphoma development in huPBL/SCID mice. For that, human peripheral blood lymphocytes (hu-PBL) from EBV-seropositive donors were inoculated intraperitoneally into SCID mouse. Twenty one out of 29 mice developed tumors in their body. Immunohistochemical staining showed that all induced tumors were LCA (leukocyte common antigen) positive, B-cell markers (CD20, CD79a) positive, and T-cell markers (both CD3 and CD45RO) negative. The tumors were diagnosed as human B-cell lymphomas by these morphological and immunohistochemical features. *In situ* hybridization exhibited resultant tumor cells with EBV encoded small RNA-1 (EBER-1). Human-derived IgG could be found in the serum of SCID mice on the 15th day following hu-PBL transplantation, and IgG levels increased as tumor grew in 6 hu-PBL/SCID chimeras. These data suggest that intraperitoneal transfer of hu-PBLs from EBV+ donors to SCID mice leads to high human IgG levels in mouse serum and B cell lymphomas.

### 3.4 Multiple Myeloma

Plasmacytoma or myeloma can be induced in BALB/c mice by pristane oil or can develop spontaneously in some mouse strains (Potter, 1982; Radl, 1981). In the former, pristane oil induces an oil granuloma characterized by a chemically-induced lymphoplasmacytic reaction. This progresses to an autonomously growing plasmacytoma with uncontrolled expression of c-MYC due to its rearrangement. Generally, these plasmacytomas secrete monoclonal immunoglobulin of the IgA isotype. Essential monoclonal gammopathies and a malignancy resembling human plasma cell myeloma may arise spontaneously in inbred mice (Radl et al., 1988; Radl, 1991).

Hence, the Radl model was produced using 5T myeloma cells that arose spontaneously in aged, inbred C57BL/KaLwRijHsd mice and is propagated by the inoculation of these myeloma cells into syngeneic mice. More specifically, in order to develop a better animal model of human myeloma bone disease, Garrett et al. (1997), have established and subcloned a cell line from this murine myeloma and found that it causes osteolytic bone lesions in mice characteristic of human myeloma bone disease. The cell line produces interleukin-6, but grows independent of exogenous interleukin-6. Mice inoculated

intravenously with the cultured cells predictably develop an identical disease to the mice injected intravenously with fresh bone-marrow-derived myeloma cells, including monoclonal gammopathy and radiologic bone lesions. They found that some of the mice became hypercalcemic, and the bone lesions are characterized by increased osteoclast activity. They found identical results when they inoculated Nu/Bg/XID mice with cultured murine myeloma cells. Because they can inoculate mice with precise numbers of cells and predict accurately when the mice will develop bone lesions, become hypercalcemic, and die, they considered it as a convenient model for determining the mechanisms by which the myeloma cells cause osteoclast activation in this model of human myeloma bone disease (Garrett et al., 1997; Radl et al., 1979, 1988).

On another approach, researchers made use of two facts:

1. Human myeloma cell lines can survive and disseminate in mice with severe combined immunodeficiency (SCID; Feo-Zuppari et al., 1992; Huang et al., 1993).
2. Fetal bone implants (SCID-hu) can sustain survival and expansion of primary human myeloma cells from untreated patients with a high success rate (Yaccoby et al., 1998).

Thus, the SCID-hu model is produced, which provides a suitable *in vivo* read out system to study human myeloma biology. Tumor self-renewal capacity can be examined in relation to maturation stage. The contributions of host accessory cells and cytokines to disease manifestation and progression can also be elucidated. It is anticipated that new treatment principles aiming, for example, to inactivate the marrow microenvironment (e.g. bisphosphonates: Aparicio et al., 1998; Shipman et al., 1997) and target neoangiogenesis (e.g. Thalidomide: D' Amato et al., 1994; Singhal et al., 1999) can be evaluated. Another animal model uses adult human bone engraftments into SCID mice. In these mice the engrafted human bone is injected and subsequently populated by fresh tumor cells. In that way a close resemblance to human multiple myeloma has been achieved (Sjak-Shie et al., 1999).

Similar to the extensively tested and validated SCID-hu system, which uses a human fetal bone (Yaccoby et al., 1998, 2002, 2006; Yaccoby & Epstein, 1999), MM cells from the majority of patients grow exclusively in the implanted bone and produce typical myeloma manifestations including stimulation of osteoclastogenesis, suppression of osteoblastogenesis, and induction of severe osteolytic bone disease (Fig. 1). Ethical and scientific concerns regarding the use of human fetal bones in the SCID-hu model of primary human myeloma prompted the researchers to develop a novel system that uses rabbit bones implanted subcutaneously in unconditioned SCID mice. Immunohistochemical analysis of the implanted bone revealed that the majority of bone marrow (BM) microenvironment cells such as blood vessels, osteoclasts and osteoblasts were of rabbit origin. The implanted bones were directly injected with myeloma cells from MM patients. Successful engraftment of unseparated BM cells from 85% of patients and CD138-selected myeloma plasma cells from 81% of patients led to the production of patients' M-protein isotypes and typical myeloma manifestations (osteolytic bone lesions and angiogenesis of rabbit origin; Fig. 1). Myeloma cells grew exclusively in the rabbit bone, but were able to metastasize into another bone at a remote site in the same mouse. Cells from patients with extramedullary disease also grew along the outer surface of the rabbit bones. This demonstrates the ability of SCID-rab model, marked by a nonmyelomatous, nonhuman, and nonfetal microenvironment, to support the growth of CD138-expressing myeloma cells. This system can now be widely used to study the biology of myeloma and its manifestations and to develop novel therapeutic approaches for this disease (Yata & Yaccoby, 2004).

Conclusively, the SCID-hu/rab xenograft model provides a system where primary human myeloma cells can be injected into either a fetal human bone or rabbit bone that is implanted subcutaneously into an immunocompromised mouse (Yaccoby et al., 1998; Yata & Yaccoby, 2004). This model (SCID-rab) has been used since its establishment for different studies, e.g. the effect of anti-DKK1 therapy on bone metabolism and tumor growth in a SCID-rab system, since DKK1 is a key player in MM bone disease and blocking DKK1 activity in myelomatous bones reduces osteolytic bone resorption, increases bone formation, and helps control MM growth (Yaccoby et al., 2007).

The most recent study from Fowler et al. (2009) describes a model of myeloma in which the host microenvironment could be modified genetically. They demonstrated 5T myeloma establishment in recombination activating gene 2 (RAG-2)-deficient mice, which have improper B- and T-cell development. Importantly, these mice can be easily bred with genetically modified mice to generate double knockout mice, allowing manipulation of the host microenvironment at a molecular level. Inoculation of 5TGM1 myeloma cells into RAG-2<sup>-/-</sup> mice resulted in myeloma development, which was associated with tumor growth within bone and an osteolytic bone disease, as assessed by microcomputed tomography (microCT), histology and histomorphometry. Myeloma-bearing RAG-2<sup>-/-</sup> mice displayed many features that were similar to both human myeloma and the original Radl 5T model. To demonstrate the use of this model, we have examined the effect of host-derived matrix metalloproteinase 9 (MMP-9) in the development of myeloma in vivo.

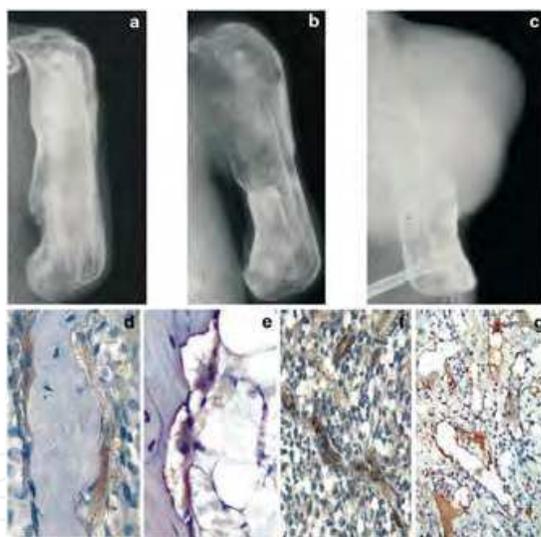


Fig. 1. Growth patterns and typical disease manifestations of myeloma cells in the SCID-rab model. (a, b) Myeloma cells from the majority of patients grew exclusively in the rabbit BM (medullary disease). Radiographs (a) before and (b) 12 weeks after myeloma PCs injection show severe resorption of the myelomatous rabbit bone. (c) Myeloma PCs taken from extramedullary disease grew also on the outer surface of the implanted rabbit bone. (d) Increased activity of multinucleate osteoclasts was detected in myelomatous rabbit bone by staining sections for TRAP. (e) These osteoclasts were of rabbit origin, as revealed by their reactivity with anti-rabbit macrophage antibody. (f, g) Myelomatous rabbit bone sections immunostained with antibody to factor VIII (f) and rabbit CD141 (g) demonstrate increased numbers of tumor-associated microvessels of rabbit origin (From Yata & Yaccoby, 2004, permission pending).

Inoculation of 5TGM1 myeloma cells into mice that are deficient in RAG-2 and MMP-9 resulted in a reduction in both tumor burden and osteolytic bone disease when compared with RAG-2-deficient wild-type myeloma-bearing mice. The establishment of myeloma in RAG-2<sup>-/-</sup> mice permits molecular examination of the host contribution to myeloma pathogenesis *in vivo*.

### 3.5 Other lymphoproliferative disorders

Primates and swine have been used as experimental animals to develop animal models for post-transplant lymphoproliferative disorder (PTLD) one of the less frequently met LPDs.

As previously mentioned, PTLD has been shown to be associated with Epstein-Barr virus (EBV) infection. Although primate animal models of PTLD and the use of molecular markers in its diagnosis had not yet been reported, Schmidtko et al. (2002) designed a study to evaluate the frequency, pathology, and molecular characteristics of PTLD in cynomolgus monkey kidney allograft recipients. Of 160 consecutive primate renal transplants performed, 5.6% developed PTLD 28-103 days after transplantation. In all cases, the lymph nodes were involved and effaced by an atypical polymorphous lymphoid proliferation of EBER+ B cells, diagnostic for PTLD. Focal staining for EBNA-2 was noted in tumor cells. In 67% (six of nine) the PTLD infiltrates were present in extra nodal sites, notably liver (56%), lung (44%), heart (44%), renal allograft (44%), and native kidney (22%). The spleen was infiltrated by PTLD cells in all four animals that had not undergone a pre-transplant splenectomy. The PTLD morphology was similar in all cases and predominantly of the polymorphous type, however, some of these showed areas that appeared minimally polymorphous. No cases of monomorphic PTLD were seen. By *in situ hybridization*, expression of the RNA product, homologous for EBV-encoded RNA (EBER) was identified in the PTLD tumor cells of all cases, indicating latent primate EBV-related infection. This report identifies a novel animal model of EBV associated PTLD in the setting of kidney transplantation, with valuable implications for managing and understanding human PTLD and oncogenesis (Schmidtko et al., 2002).

Barth et al. (2009) developed another non-human primate facial composite tissue allotransplantation model to investigate strategies to achieve prolonged graft survival and immunologic responses unique to these allografts. For this reason, composite facial subunits consisting of skin, muscle, and bone were heterotopically transplanted to mixed lymphocyte reaction-mismatched Cynomolgus macaques. Tacrolimus monotherapy was administered via continuous intravenous infusion for 28 days then tapered to daily intramuscular doses. They concluded that Tacrolimus monotherapy provided prolonged rejection-free survival of composite facial allografts in this non-human primate model but was associated with the development of a high frequency of donor-derived PTLD tumors. The transplantation of a large volume of vascularized bone marrow in composite tissue allografts may be a risk factor for PTLD development.

A high incidence of a PTLD is observed in miniature swine conditioned for allogeneic hematopoietic cell transplantation using a protocol involving T-cell depletion and cyclosporine therapy. Cho et al. (2004), designed a study to assess contributing factors to disease development. Forty-six animals were studied including 12 (26%) that developed PTLD. A number of risk factors for PTLD were examined, including degree of immunosuppression, degree of MHC mismatch and infection by a porcine lymphotropic herpesvirus (PLHV-1). Flow cytometry was used to measure host and donor T- and B-cell

levels in the peripheral blood. Porcine lymphotropic herpesvirus viral load was determined by quantitative PCR. Animals developing PTLD had significantly lower levels of T cells on the day of transplant. Cyclosporine levels did not differ significantly between animals with and without PTLD. Animals receiving transplants across a two-haplotype mismatch barrier showed an increased incidence of PTLD. All animals with PTLD had significant increases in PLHV-1 viral loads. Porcine lymphotropic herpesvirus viral copy numbers remained at low levels in the absence of disease. The availability of a preclinical large-animal model with similarities to PTLD of humans may allow studies of the pathogenesis and treatment of that disorder.

Spleen transplantation (SpTx) was also performed in miniature swine across full major histocompatibility complex barriers to study the tolerogenic effect of the spleen (Dor et al., 2004). This study described the development of PTLD after allogeneic SpTx. Recipient pigs underwent whole body irradiation (100 cGy), thymic irradiation (700 cGy), and native splenectomy (day 0), and received a 45-day course of intravenous cyclosporine (trough level 400-800 ng/ml). After SpTx, two of seven pigs developed PTLD (1 donor-type, 1 host-type). These two pigs had greater T cell depletion and higher trough levels of cyclosporine. Early changes that occurred prior to the development of clinical features of PTLD were increased porcine lymphotropic herpesvirus-1 viral loads in blood and tissues, and increased numbers of leukocytes, B cells, and total serum IgM. PTLD can occur after allogeneic SpTx in swine. This model may be useful in studies of the pathogenesis of PTLD.

In another study using miniature swine (Doucette et al., 2007) the Porcine lymphotropic herpesvirus-1 (PLHV-1), a gamma-herpesvirus related to Epstein-Barr virus (EBV) was associated with development of PTLD following allogeneic stem cell or spleen transplantation. Oligonucleotide microarrays were designed based on known open reading frames (ORFs) of PLHV-1. Expression was compared by cohybridization of cDNA from lymph nodes of PLHV-1+ swine after allogeneic spleen transplantation between either: 1) PTLD-affected and PTLD-unaffected swine; or 2) PTLD-affected swine vs. samples from the same animal prior to diagnosis. In PTLD-affected animals, consistent upregulation (nine ORFs) and downregulation (four ORFs) of PLHV-1 mRNA was observed in comparison to those without PTLD. No differences in gene expression were discovered at the time of clinical PTLD diagnosis compared to six to nine days prior to diagnosis in the same animals. This model provides insights into the pathogenesis of PTLD and, by extension, potential diagnostic and therapeutic tools for human EBV-associated PTLD.

#### **4. Waldenström's macroglobulinemia**

This disease was first identified by J. Waldenström when he reported two patients with a syndrome of oronasal bleeding, lymphadenopathy, an elevated sedimentation rate, hyperviscosity, normal bone films, cytopenias and a bone marrow with a predominantly lymphoid infiltrate (Waldenström, 1944).

In the Revised European-American Lymphoma (REAL) classification, Waldenström macroglobulinemia (WM) has become viewed as a distinct clinicopathological entity, and is defined largely as a lymphoplasmacytic lymphoma (LPL); (Harris et al, 1994). The Second International Workshop on Waldenström macroglobulinemia attempted to refine further the working definition of the disease within the context of a LPL (Owen et al., 2003). For review, see Ansell et al., 2010.

Despite these efforts, the debate remains within the hematological and hematopathological communities with respect to nosology. These issues of definition have and continue to affect the interpretation of results within and across clinical trials as well as in basic and epidemiological research (Fonseca & Hayman, 2007). The same researchers (Fonseca & Hayman, 2007) used the definition of WM as proposed at the Second International Workshop on WM, with the exception that the original criterion of the presence of any degree of marrow involvement with a lymphoplasmacytoid infiltrate has been modified to allow a distinction to be made between an immunoglobulin (Ig) M monoclonal gammopathy of undetermined significance (MGUS) and WM. This change is based on analyses that have established the prognostic relevance and statistically significant survival differences among IgM MGUS (<10% marrow lymphoplasmacytic infiltrate) and symptomatic/smouldering WM and symptomatic/active WM ( $\geq 10\%$  lymphoplasmacytic marrow infiltrate; usually intertrabecular).

Thus, Waldenström macroglobulinemia is a B-cell lymphoproliferative disorder characterized by a lymphoplasmacytic infiltration in the bone marrow or lymphatic tissue and a monoclonal immunoglobulin M protein (IgM) in the serum (Dimopoulos et al., 2005; Owen et al., 2003). Is a rare hematological neoplasm with an overall incidence of approximately 5 cases per 1 million persons per year, accounting for 1-2% of haematological malignancies (Groves et al., 1998; Herrinton & Weiss, 1993).

The median age at diagnosis varies between 63 and 68 years, and most patients (55%-70%) with newly diagnosed disease are men (Dimopoulos et al., 2000). The incidence of Waldenström macroglobulinemia is highest among white people and is rare in other population groups (Benjamin et al., 2003). More specifically, WM is rare in Blacks, who represent only 5% of cases, and it is also rare in those of Mexican-Mestizo descent (Groves et al., 1998; Herrinton & Weiss, 1993; Ruiz-Arguelles et al., 2000). To date, there is no compelling evidence to link WM to specific occupational or environmental exposures, tobacco or alcohol use (Linnet et al., 1993).

The aetiology of WM remains unknown. It appears to be primarily a sporadic disease, although there are multiple reports of familial clustering (Blattner et al., 1980; Brown et al., 1967; Elves & Brown, 1968; Getaz & Staples, 1977; McMaster et al., 2005; Ogmundsdottir et al., 1999; Renier et al., 1989; Treon et al., 2006). McMaster et al. (2006) performed a genomewide linkage analysis in 11 high-risk families with WM that were informative for linkage (including a total of 122 individuals with DNA samples). The strongest evidence of linkage was found on chromosomes 1q and 4q (McMaster et al., 2006). Treon et al. (2006) evaluated 257 patients with previously untreated WM and found that 18,7% had at least one first-degree relative with either WM or another B-cell disorder. In addition, those with a familial history had higher percentages of bone marrow involvement, were diagnosed at a younger age, and were more likely to have higher IgM levels upon initial presentation (Treon et al., 2006).

The greatest risk factor for the development of WM is that of having an IgM MGUS. These patients have 46 times greater risk of developing WM than the general population (Kyle et al., 2002). Factors affecting the progression from IgM MGUS to WM are unknown. Infiltration of the bone marrow and extramedullary sites by malignant B cells and elevated IgM levels account for the symptoms associated with this disease. Patients may develop constitutional symptoms, pancytopenia, organomegaly, neuropathy, and symptoms

associated with immunoglobulin deposition or hyperviscosity (Dimopoulos et al., 2000; Vijay & Gertz, 2007). However, symptoms vary considerably in individual patients. Although some patients present with the aforementioned symptoms, many are asymptomatic at the time of diagnosis.

Waldenström macroglobulinemia is incurable with current therapy, and half of the patients die of disease progression; median survival is approximately 5 years (Dimopoulos et al., 1999). This disease is diagnosed in many patients at an advanced age, and thus approximately half of the patients die of causes unrelated to Waldenström macroglobulinemia. Because the disease is incurable and the clinical presentations, comorbidities, and causes of death vary substantially, the decision to treat patients and the choice of treatment can be complex. A number of consensus meetings have listed reasonable treatment options (Gertz et al., 2003; Treon et al., 2006; Dimopoulos et al., 2009) but the physician is still faced with a difficult treatment decision in a patient with an uncommon disease.

## 5. Animal models of Waldenström's macroglobulinemia

In 2003, the Wayne State University Waldenström's Macroglobulinemia xenograft model in mice with severe combined immune deficiency (WSU-WM-SCID) was developed (Al-Katib et al., 2003). The WSU-WM-SCID is a model of a more aggressive and resistant WM usually seen toward the late stages of disease. It is, therefore, a particularly useful tool in developing new therapeutic strategies for the more aggressive WM, including targeted therapy, which exploits unique molecular characteristics of tumor cells. The WSU-WM-SCID is the first preclinical animal model available for this disease. It is based on a permanent, EBV-IgM $\lambda$  cell line (WSU-WM) established from a patient with a 10-year history of Waldenström's macroglobulinemia. These cells are CD5(-)CD10(+)CD19(+)CD20(+)CD22(+) and have t(8;14) (q24;32), t(12;17) (q24;q21), 2p-. WSU-WM cells also express DNA topoisomerase II (alpha and beta), and are bcl(2)(+)bcl(XL)(+)bax(-). Although the tumor has aggressive biological behavior with c-myc-IgH rearrangement, it has retained the salient features of WM. The breakpoint on 8q24 is downstream of c-myc exon 3, which is not usual for Burkitt-type breakpoints. WSU-WM cells also express both secretory (s(u)) and membrane (m(u)) IgM mRNA and secrete IgM in culture supernatant. Histologically, WSU-WM-SCID xenograft tumors have lymphoplasmacytoid morphology. These features indicate biological, but not histological evolution.

In 2005, Tassone et al., developed a novel *in vivo* model of human WM in severe combined immunodeficient (SCID) mice implanted with human fetal bone chips (SCID-hu mice) into which WM cells from patient bone marrow are engrafted directly into the human bone marrow (huBM) microenvironment. WM cells in SCID-hu mice produced human monoclonal paraprotein (immunoglobulin M [IgM] and/or kappa or lambda chain) detectable in mice sera. Immunohistochemical analysis of human bone retrieved from SCID-hu mice showed infiltration with CD20+, IgM+, and monotypic light chain+ lymphoplasmacytic cells. Mast cells were observed to be associated with the infiltrate in these sections. Treatment of SCID-hu mice bearing WM with rituximab induced tumor regression, associated with a decrease in serum paraprotein. This model, therefore, recapitulates the *in vivo* biology of WM and allows the study of novel investigational drugs targeting WM cells in the huBM milieu.

Model cell lines are essential tools for investigating the biology and therapeutics of cancer. Approximately 1500 human hematopoietic neoplastic cell lines have been described,

covering most major disease entities. Waldenström's macroglobulinemia (WM) is a rare incurable hematological neoplasm from which four cell lines have been derived.

In 2007 a cell line, the BCWM.1 cell line (Ditzel Santos et al., 2007), which was derived from the long-term culture of CD19(+) selected bone marrow lymphoplasmacytic cells isolated from an untreated patient with WM. BCWM.1 cells morphologically resemble lymphoplasmacytic cells (LPC) and propagate in RPMI-1640 medium supplemented with 10% fetal bovine serum. Phenotypic characterization by flow cytometric analysis demonstrated typical WM LPC characteristics: CD5(-), CD10(-), CD19(+), CD20(+), CD23(+), CD27(-), CD38(+), CD138(+), CD40(+), CD52(+), CD70(+), CD117(+), cIgM(+), cIgG(-), cIgA(-), ckappa(-), clambda(+), as well as the survival proteins APRIL and BLYS, and their receptors TACI, BCMA and BAFF-R. Enzyme-linked immunosorbent assay studies demonstrated secretion of IgMlambda and soluble CD27. Karyotypic and multicolor fluorescence in situ hybridization studies did not demonstrate cytogenetic abnormalities. Molecular analysis of BCWM.1 cells confirmed clonality by determination of IgH rearrangements. Inoculation of BCWM.1 cells in human bone marrow chips implanted in severe combined immunodeficient-hu mice led to rapid engraftment of tumor cells and serum detection of human IgM, lambda, and soluble CD27. These studies support the use of BCWM.1 cells as an appropriate model for the study of WM, which in conjunction with the severe combined immunodeficient-hu mouse model may be used as a convenient model for studies focused on both WM pathogenesis and development of targeted therapies for WM.

In 2008, Drexler et al., summarized on the existence of three cell lines, that although are currently used as *in vitro* models, none convincingly pass muster. Mindful that candidate tumor cell lines sometimes arise spuriously by viral immortalization of bystander cells, they reviewed the extent to which WM cell lines portray established disease features *in vitro*. At closer inspection, it seems that none convincingly displays morphological, immunophenotypic, genotypic or biological features characteristic of WM. Rather it appears that two cell lines (WM1 and BCWM.1) are most probably Epstein-Barr virus-immortalized B-lymphoblastoid cell lines, derived from bystander B-cells. The third cell line (WSU-WM) carries the most common cytogenetic hallmark of Burkitt lymphoma, namely t(8;14)(q24;q32), while none have been shown to carry chromosome 6 deletions recently demonstrated as indicative of disease progression in this entity.

Recently, Hodge et al. (2011) described the establishment of a new WM cell line, MWCL-1. Comprehensive genetic analyses have unequivocally confirmed a clonal relationship between this novel cell line and the founding tumor. MWCL-1 cells exhibit an immunophenotype consistent with a diverse, tumor clone composed of both small B lymphocytes and larger lymphoplasmacytic cells and plasma cells: CD3<sup>-</sup>, CD19<sup>+</sup>, CD20<sup>+</sup>, CD27<sup>+</sup>, CD38<sup>+</sup>, CD49D<sup>+</sup>, CD138<sup>+</sup>, cIgM<sup>+</sup>, and κ<sup>+</sup>. Cytogenetic studies identified a monoallelic deletion of 17p13 (TP53) in both the cell line and the primary tumor. Direct DNA resequencing of the remaining copy of TP53 revealed a missense mutation at exon 5 (V143A, GTG>GCG). In accordance with primary WM tumors, MWCL-1 cells retain the ability to secrete high amounts of IgM protein in the absence of an external stimulus. The genetic, immunophenotypic, and biologic data presented here confirm the validity of the MWCL-1 cell line as a representative model of WM.

Dr. Janz at Department of Pathology at University of Iowa is currently developing a mouse model of Waldenström's macroglobulinemia. An immunocompetent, transgenic mouse

model of human WM that will be useful for preclinical testing of WM drug candidates. Transgenic mouse models of human cancer are experimental model systems that rely on laboratory mice that have been genetically manipulated to render them prone to neoplasms that accurately recapitulate important features of their human cancer counterparts. Model systems of this sort: enable researchers to study the onset and progression of cancer in ways that cannot be pursued in human beings; advance our understanding of the molecular genetic and biological events that contribute to the development and spread of cancer cells; and provide a valuable preclinical platform for evaluating new approaches to treat and prevent cancer in patients. The latter is particularly important in circumstances in which drug testing requires an intact, immunocompetent animal that is able to produce the same kind of tumor microenvironment and recruit the same types of tumor bystander cells commonly found in human patients. To give but one example, therapeutic antibodies target cancer cells by recruiting normal immune cells to the site of attack; thus, the preclinical testing of these antibodies requires strains of laboratory mice that have a normal, fully functioning immune system. To that end, Janz and collaborators are generating a designer model of human WM designated C.IL6/BCL2/AID<sup>null</sup>. This model combines three crucial pathogenetic factors of human WM – namely the B-lymphocyte growth, differentiation and survival factor IL-6, the cellular oncoprotein BCL-2, and the inability of WM cells to perform immunoglobulin isotype switching (AID<sup>null</sup>) – on the genetic background of BALB/c (abbreviated as C). Strain C mice are highly susceptible to malignant B-lymphocyte transformation (Diagram 1; [http://www.medicine.uiowa.edu/Pathology/site/research/janz/res\\_projects.html](http://www.medicine.uiowa.edu/Pathology/site/research/janz/res_projects.html); selected publications of Dr. Janz: de Jong & Janz, 2010; Park et al., 2005).

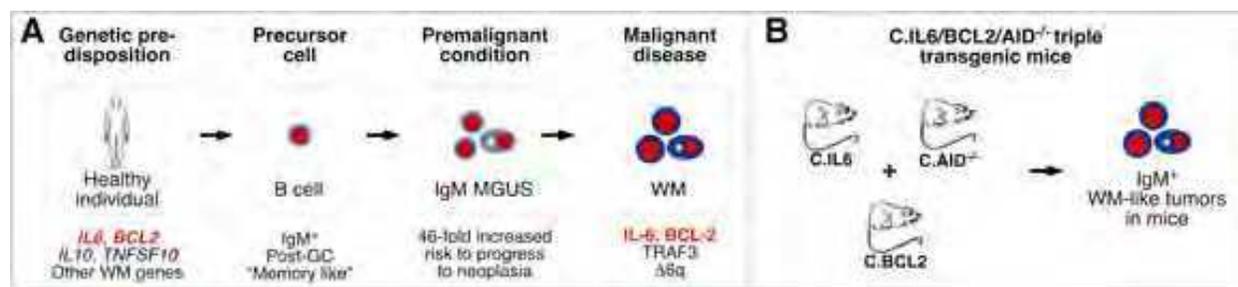


Diagram 1. Schematic overview of the pathogenesis of lymphoplasmacytic lymphoma (LPL)-WM (panel A) and transgenic mouse strains that Janz et al. propose to use for modeling human LPL-WM in mice (panel B).

(A) *IL6* and *BCL2* have been identified as "WM genes" – genes that confer genetic proclivity to the disease (left). Additionally, IL-6 and BCL-2 are major player in the LPL-WM cells (right)

(B) All strains are on the same genetic background of BALB/c (C), an important precondition for intercrossing the various transgenes without jeopardizing crucial practical issues of this project, such as the ability to adoptively transfer fully transformed tumor cells or premalignant B-lineage cells from transgenic mice. We hypothesize that strain C.IL6/BCL2/AID<sup>-/-</sup> mice will develop IgM<sup>+</sup> WM-like tumors.

### 5.1 Waldenström's macroglobulinemia in NOD/SCID mice

Although important advances have been made in the classification of lymphomas, the remaining discrepancies in WM definition reflect the fact that the pathogenesis of this disease remains largely unknown.

As stated in the previous paragraph, the establishment of reliable animal models will significantly enhance research in dissecting the complicated pathogenesis of WM. In addition, suitable animal models may be used to assess the efficacy of existing treatments and develop novel therapeutic strategies (Al-Katib et al., 1993; 2003). Severe combined immunodeficient (SCID) mice injected with subcutaneous xenografts of neoplastic cells were originally used to study WM. However, the usefulness of these models is limited because they do not recapitulate typical features of WM, such as bone marrow localization. Recently developed SCID mouse WM models overcome this drawback, by utilizing subcutaneous implants of human fetal bone chips. The subsequent injection of bone marrow (BM) aspirates from WM patients directly into the fetal bone implants resulted in successful WM cell engraftment in 69% of animals (Tassone et al., 2005). Although this model offers a potential advantage in that human bone is used, fetal bone, which is at a state of rapid growth, clearly differs from adult bone. It is apparent that there are differences between the bone marrow of a newborn and an adult bone marrow, including apoptotic cells, T cells, B cells and macrophages, developing the microenvironment where the WM cells are being hosted. Not to mention the fact that human bone is made of cells forming temporary anatomical structures, called basic multicellular units that execute bone remodeling, which change with ageing of humans (Seeman, 2008).

Our study was undertaken with the aim to develop a novel non-obese SCID (NOD-SCID) mouse model of WM. For that, pairs of bone particles derived from adult humans were successfully implanted intramuscularly in mice. Each mouse was implanted with a bone fragment taken from a neoplastic disease-free individual in the one hind limb and with a different biopsy taken from a WM patient, in the other. IgM producing neoplastic cells not only retained viability in the bone marrow of the WM bone biopsy but also metastasized to the normal bone marrow of the distant bone implant. The mouse model reported here improves on existing models of WM by recapitulating the adult human bone marrow microenvironment of abnormal WM neoplastic cells.

For this reason, twenty-nine NOD-SCID mice (Charles River Laboratories, France) were used. The animals were housed in static microisolator cages at the bio-containment animal Research Facility of the G. Papanicolaou General Hospital. All experimental procedures and protocols were in accordance with the European Council Directive 86/609 as well as the national and institutional guidelines for animal care.

Cancellous bone core fragments ranging from 16 to 22 mm<sup>3</sup> in size were obtained from the femoral head of neoplastic disease-free adult humans during hip arthroplasty or hemiarthroplasty. Bone fragments were subsequently implanted into the right or left hindlimb muscles of 6 to 8 weeks-old mice (n=23), weighing 25 - 30 grams, as previously described (Tsingotjidou et al., 2001). To test viability of implanted tissue, implants were retrieved from 9 mice that were sacrificed at 4 (n=3), 8 (n=3) and 12 (n=3) weeks post-implantation. Based on previous studies (Boynton et al., 1996) in order to detect hematopoietic cells of human origin mouse anti-human CD45 antibody (1:500; Dako, Carpinteria, CA) was used. Eight to twelve weeks following first bone implantation, the non-implanted hindlimb of the remaining 12 mice was also engrafted with a human bone biopsy taken from WM patients (see flow chart; Diagram 2). For that, bone marrow core needle biopsies (bone biopsies) were obtained from the posterior iliac crest of five patients with active WM during scheduled clinical visits. The biopsy was maintained in RPMI

medium until its use. Typically, two implants were produced from each bone biopsy. In one case, however, a single WM bone biopsy was large enough to be the source of 6 implants. All human bone biopsy donors signed Institutional Review Board-approved informed consent forms. Control mice received no implant (n=3) or were implanted only, either with non-WM (n=2) or with WM (n=3) human bone fragments (Diagram 2).

To detect human IgM in the serum of mice, thus providing evidence for presence of human IgM-secreting B-cells or WM cells, mouse blood was collected from the orbital sinus of each mouse monthly during the experiment. Blood serum was serially tested for circulating IgM by ELISA (Diagnostic automation Inc., Calabasas, CA, USA).

Mice were sacrificed at different time points ranging from 1 to 5 months following the implantation of WM bone biopsies (Diagram 2). At necropsy, tissues including femur, tibia, brain, liver, spleen, lungs, kidneys and the WM and non-WM bone grafts were collected and fixed in formalin. Decalcified bones together with other formalin-fixed tissues were processed routinely, embedded in paraffin, sectioned at 5  $\mu$ m, and stained with haematoxylin and eosin (HE) or immunohistochemistry (IHC). The latter was performed with mouse monoclonal antibodies directed against human CD20 (Biogenex, San Ramon, CA, USA), IgM (Cell Marque, Rocklin, CA, USA), and  $\kappa$  and  $\lambda$  light chains (Novocastra Laboratories, Newcastle-upon-Tyne, UK). Antigens were retrieved with microwave heating in citrate buffer (pH 6) for CD20, or with proteinase K (DAKO, Carpinteria, CA, USA) digestion for 6 minutes at room temperature for IgM and  $\kappa$  or  $\lambda$  chains. Poly-HRP goat anti-mouse IgG (Chemikon, Temecula, CA, USA) was used as secondary antibody. Signal was detected with diaminobenzidine and tissues were counterstained with haematoxylin. Wild type mouse colon along with mesenteric lymph nodes sections were used as negative tissue controls, whereas positive controls were procured from bone biopsy of WM patients not involved in the study. Irrelevant mouse antibodies were used instead of primary antibodies for negative staining controls.

We (Tsingotjidou et al., 2001) and others (Yonou et al., 2001) have previously shown that adult human bone retains viability after engraftment subcutaneously or within the skeletal muscles of immunocompromised mice. In the present study, we first sought to determine whether the above-mentioned model of bone engraftment was successfully reproduced. For that, adult human cancellous bone intramuscular implants were recovered from control SCID mice at 4, 8 or 12 weeks post-implantation for histological evaluation. At four weeks, specimens showed multifocal necrosis and fibrosis. The surface of the human bone was lined by small numbers of osteoblast-like cells and minute amount of newly synthesized osteoid. In contrast, all human bone implants from intramuscularly implanted mice were largely normal at the 8-week time-point. Viable osteocytes and increased numbers of osteoblasts forming adequate amounts of osteoid were evident. However, rare foci of remaining osteonecrotic and fibrotic changes were noticed. At 12 weeks after implantation, the histology of intramuscularly implanted bone was completely restored. The population and spatial distribution of osteoblasts, the amounts of newly formed bone matrix, the restored numbers of viable osteocytes (Fig. 2A) and the presence of active bone marrow cavities (Fig. 2B) were all consistent with normal bone histology. The human origin of haematopoietic cells populating the bone marrow of implants was confirmed by IHC using an antibody against human CD45 that shows no cross-reactivity with mouse CD45 protein (Tsingotjidou et al., 2001; Boynton et al., 1996). No human lymphoid cell was found in

murine tissues examined. These results suggest that human bone implant was maintained and thrived in the mouse skeletal muscle environment.

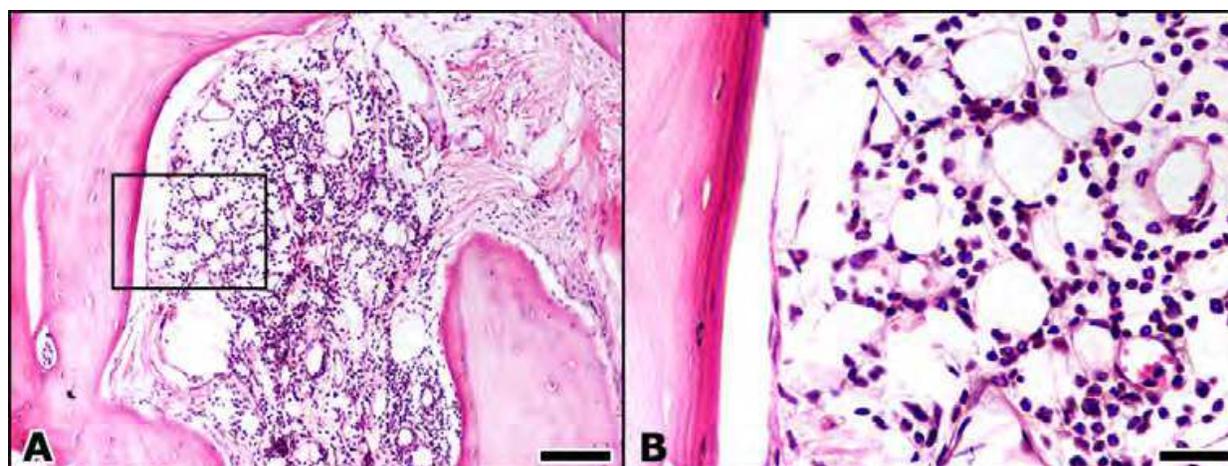


Fig. 2. Non-WM human bone implanted in the hind limb muscles of a NOD-SCID mouse at 12 weeks post-implantation. Higher magnification of the boxed area in A is shown in B. Histology of the human bone is restored. Note new bone formation with viable osteoblasts and bone marrow reconstitution. Haematoxylin & Eosin. Bars, A: 100 μm; B: 25 μm.

Three animals bearing WM bone biopsy alone were sacrificed at 1 ( $n=1$ ), 2 ( $n=1$ ) and 3 ( $n=1$ ) months post implantation (Diagram 2). In all three animals, the bone marrow was diffusely infiltrated by neoplastic cells that had lymphocytic, plasma cell or lymphoplasmacytic phenotype. Tumor cells were often arranged in discrete nodules. Reactive hyperplasia of tumor-associated mast cells was common. Necrotic areas were evident at 1 month post implantation, feature that was diminished afterwards (at 2 and 3 months post implantation).

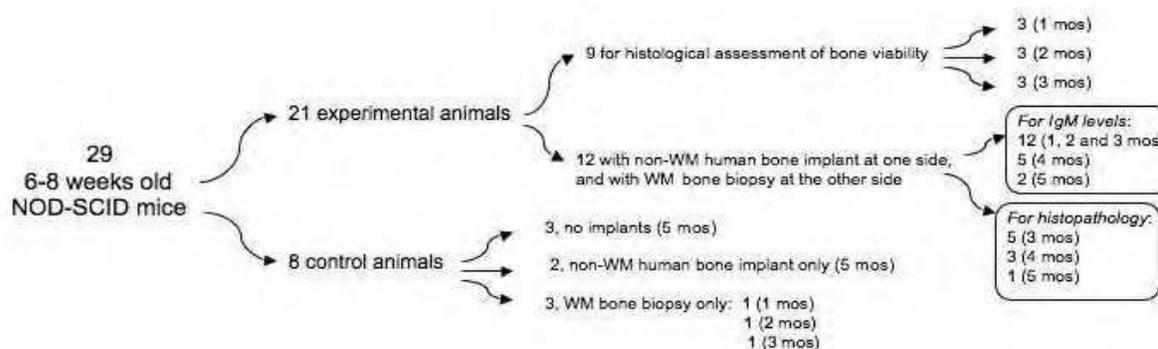


Diagram 2. Flow chart of the experimental design; Time at parentheses indicates the sacrifice point, mos=months

Immunohistochemically, neoplastic cells were CD20 positive. IgM-positive neoplastic cells were also identified in large numbers. Both CD20 and IgM antibodies used for this purpose was against human lymphocytes and IgM. These mice also had elevated IgM in serum at one month following the intramuscular implantation of the biopsy. However, two months post-implantation, blood IgM was moderately-to-markedly reduced. In a single mouse IgM fell to non-detectable levels, while in the remaining moderately elevated IgM was detectable

up to three months following the WM bone biopsy implantation, without reaching, however, the high IgM levels encountered at one month post-implantation (Tsingotjidou et al., 2009). Control animals that received no implant ( $n=3$ ) or were implanted only with non-WM ( $n=2$ ) showed no elevation in serum IgM throughout the duration of experiment. Upon histological and IHC examination control mice had no detectable lymphocytes or plasma cells in all tissues examined.

Twelve animals ( $n=12$ ) implanted with both WM and non-WM bones were monitored for serum IgM levels throughout the experiment. Necropsies were performed in nine of those (9/12) at 3 ( $n=5$ ), 4 ( $n=3$ ) and 5 ( $n=1$ ) months following implantation of WM bone biopsies (Diagram 2). Histopathological and immunohistochemical analysis of WM implants in these mice ( $n=9$ ) yielded similar results as those described in the control mice bearing WM bone biopsy alone ( $n=3$ ), namely a CD20+, IgM+ neoplastic cell infiltrate. These results indicated that survival of neoplastic cells in this experimental model could be prolonged for at least 5 months post implantation. Interestingly, histopathology and IHC analysis of the controlateral, non-WM implant of 4 out of 9 mice revealed that the bone marrow was infiltrated by large numbers of neoplastic cells (Fig. 3). In order to confirm the validity of our findings, clonality by staining against  $\kappa$ - and  $\lambda$ - light chains was documented in two, randomly selected animals (W6 and W10). Cytoplasmic immunolabelling for  $\kappa$ - but not for  $\lambda$ -light chains was evident and restricted specifically and exclusively within the plasmacytic component of the tumor (Fig. 4). Thus, light chain predominance confirmed the clonality of neoplastic cells. Sparsely dispersed IgM positive cells were found in the bone marrow of tibia and femur of one animal (W10). Diffuse infiltration of WM cells was also seen in the liver and kidney of another animal (W44).

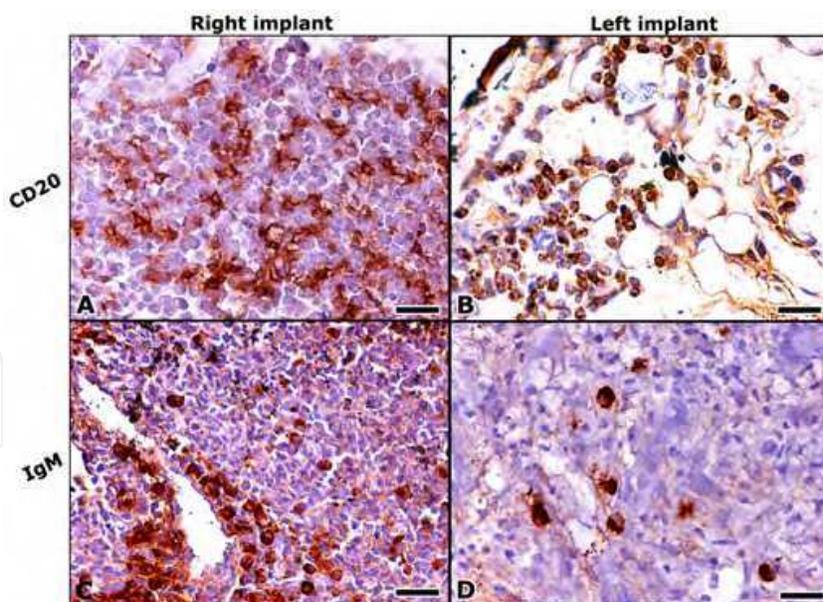


Fig. 3. CD20-specific (A and B) and IgM-specific (C and D) immunohistochemistry of human bone implants. Large numbers of human CD20-positive (A) and IgM-positive (C) WM cells remain viable in the bone marrow of the WM bone biopsy implanted in NOD-SCID mice. CD20-positive (B) and IgM-positive (D) cells could also be detected at the non-WM human bone that was distantly implanted in the same mice. Haematoxylin counterstain, DAB chromogen. Bars: 25  $\mu$ m.

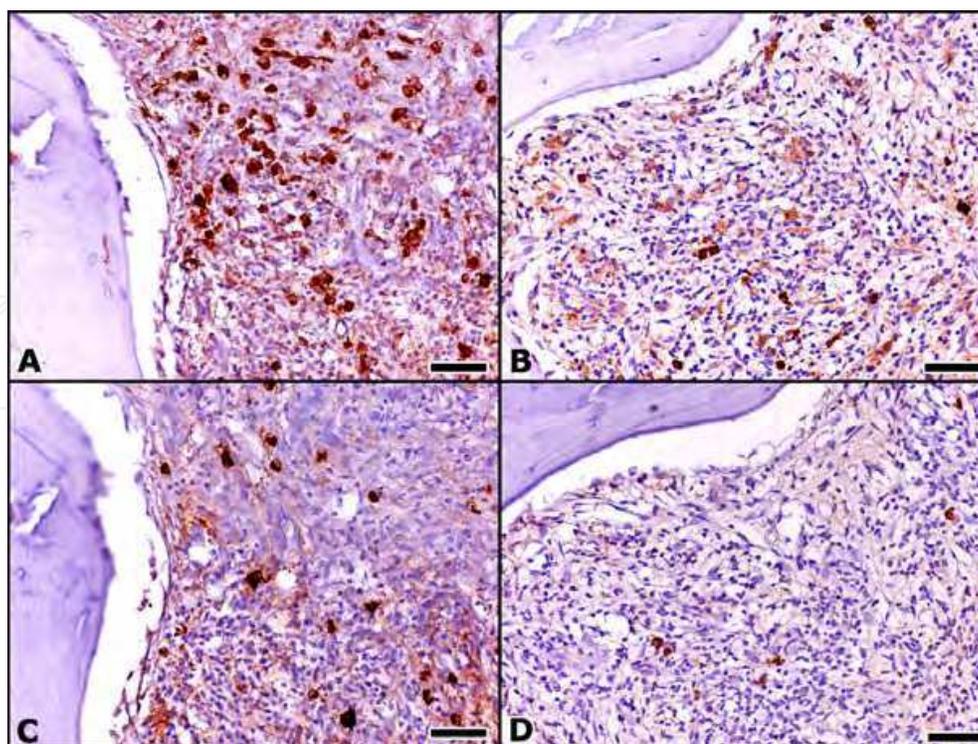


Fig. 4.  $\kappa$  (A and B) and  $\lambda$  (C and D) chain immunophenotyping of neoplastic cells performed in consecutive histological sections taken from both WM (A and C) and non-WM (B and D) bone implants of the same mouse.  $\kappa$ -chain-positive cell predominance in both implants provided evidence for the clonality of neoplastic cells. Haematoxylin counterstain, DAB chromogen. Bars: 50  $\mu$ m

Serum IgM values in all mice implanted with both WM and non-WM bone biopsies ( $n=12$ ) correlated with histopathological observations and IHC analysis for neoplastic cell density and metastatic growth. Indeed, mice with increased neoplastic cell burden and contralateral bone implant metastasis (W10, W44, W52 and W6) had increased IgM values in their blood serum (Tsingotjidou et al., 2009). In contrast, mice with sparse neoplastic cells in the WM implant and no evidence of metastasis (W45, W51 and W50) had low or non-detectable levels of serum IgM. It is interesting that 2 out of these 3 mice received implants originating from patients that had the two lowest percentages of bone marrow infiltration by neoplastic cells (20% and 33%) among human bone biopsy donors in this study. In a single mouse (W46), a sudden abolishment of serum IgM levels was observed at the 4th month post implantation of the WM bone (Tsingotjidou et al., 2009). Histological evidence of necrosis found in both implanted bone fragments examined, however, explained this abnormality.

Overall analysis of the monthly records of serum IgM values of mice used in this study reveals that after the second month, there is an overall progressive rise of IgM during months 3 and 4 post implantation of the WM biopsy (Tsingotjidou et al., 2009).

Taken together these results suggest that the WM neoplastic cells in the bone marrow of adult human bone implanted in SCID mice not only survive and grow but also are capable of producing IgM and metastasize (Tsingotjidou et al., 2009). The relatively high value of IgM obtained one month after implantation is considered an artifact attributable to cell damage, as discussed below.

This study described a novel NOD-SCID mouse model of WM. Using the SCID mouse as a vehicle of two human adult bone intramuscular implants we demonstrated that IgM producing WM neoplastic cells retain viability in the bone marrow of the implant originating from WM patients. Interestingly, neoplastic cells not only grew but also metastasized to the normal bone marrow of the second distant bone implant (Fig. 3).

A significant drawback in understanding pathogenesis of WM and developing novel therapeutics is the lack of animal models that recapitulate most features of human WM. Hence, several studies have focused in the development of animal models to study this rare but incurable neoplastic disease. In the past, attempts to grow WM cells *in vitro* or engraft them in immunocompromised mice have failed (Al-Katib et al., 2003). This probably was due to the lack of human bone marrow stromal cells, which are important elements of tumor microenvironment and facilitate both survival and proliferation of WM cells. Recently, however, important advances have been made utilizing SCID mice that were implanted with human fetal bone chips. The bone marrow of fetal bone implants successfully supported the survival and growth of WM cells derived either directly from patients (Tassone et al., 2005) or from a WM cell line that was established for that purpose (Santos et al., 2007). Both those experimental models are important, since they recapitulate a typical feature of the human disease, which is the bone marrow microenvironment-dependent growth of WM cells. However, the natural niche of WM cells is the adult and not the fetal bone marrow that both these studies utilized for the engraftment of malignant cells. Adult and fetal bone marrow may differ in the pattern of growth stimuli imparted to WM cells. Although these differences cannot be elucidated, without elaborate research, the murine model of WM proposed in the present study overcomes this skepticism by simply using adult human bone implants.

An interesting observation of this study was that mice implanted with a WM bone fragment, showed elevation of blood IgM one month following the implantation. This peak in IgM levels observed at the first month coincided with the period of maximum damage to the implanted bone particle (Tsingotjidou et al., 2001). This particular elevation, however, may not reflect an active secretion of IgM from WM cells, but rather be due to an initial phase of ischaemic injury leading to WM cell necrosis and subsequent IgM leak. Indeed, histological studies of the human bone implants performed in the present study matched our previous observations (Tsingotjidou et al., 2001), which indicated that 8-12 weeks are needed before implanted bones become adequately vascularized, and restore their pre-transplant normal histology. A similar phenomenon of IgM release is well characterized in human patients following effective anti-neoplastic treatment, due to WM cell damage (Treon et al., 2004). For that reason, only IgM serum values obtained from the second month onwards were taken into consideration and were used as reliable indicators of disease burden of the implanted mice.

The wide range of IgM levels observed in mice of our experiments mimic analogous findings in humans. Accumulated data suggest that the levels of IgM production are highly variable among WM patients. Therefore, such data are valuable for follow-up examinations of individual patients and do not represent a universal indicator of WM burden. Irrespectively of initial IgM levels, however, most mice used in this study showed a similar pattern of IgM fluctuation over time. Following an elevation of IgM at the first month and a subsequent reduction at the second month, which can be considered baseline, there was a progressive rise of IgM during months 3 and 4 post implantation of the WM biopsy.

An interesting feature of the WM murine model presented here is the observed metastasis of the malignant cells. In approximately half of mice implanted with both WM and non-WM bone implants, WM cells metastasized from the affected implant to the healthy one, which was located in the contralateral hindlimb. Human bone, albeit from unrelated individual, was colonized preferentially, since metastasis to murine bone was observed only in a single mouse. Accordingly, existing murine models of WM show either no (Santos et al., 2007) or rare (Tassone et al., 2005) metastases to murine tissues. It is possible, that WM cells engrafted in mice could infrequently acquire novel properties that allow them to traffic, home and survive in murine bone marrow or other tissues. Obviously, accumulated genetic abnormalities driven by a physical selection process, may result in WM cells able to survive in the murine microenvironment. However, these genetic alterations render these cells less appropriate to model metastatic phenomena occurring in human WM, which is predominately an indolent disease. The murine model developed in the present study overcomes inherited disadvantages of existing WM *in vivo* models and better recapitulates metastasis, since it is supporting relocation of primary WM cells derived from patients from one human bone implant to another.

## 6. Conclusion

The use of bone biopsies taken from different WM patients as implants in SCID mice appears as highly attractive biological system to study aspects of the human disease. The quantitative limitation of harvesting BM particles from patients restricts the possibility to produce large highly homogeneous experimental groups of mice, given the variability of biological behaviour of WM among patients. This fact is exemplified by the results of the present study. Indeed, high levels of serum IgM and infiltration of the contralateral non-WM bone biopsy, by WM cells did not occur consistently in all mice. Hence, we reason that this model may not be suitable for screening specific therapeutic factors of WM. For such studies, the adult bone implant approach might still be considered if the healthy bone was artificially populated with WM cell line. On the other hand, the experimental design used in the present study may be useful for studying the pathogenesis of WM and particularly the interaction with the bone marrow stroma as it exists in the adult bone marrow. It may also be an ideal setting for studying the disease in long term, since the model allows indolent growth in mice, and consequently to assess the pathophysiology of epiphenomena such as neuropathy (work in progress). It is believed that the experimental setting as presented here will contribute to the unraveling of the etiology and pathogenesis of WM to the benefit of the patients.

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## 8. References

Al-Katib, A.R., Mohammad, R., Hamdan, M., Mohamed, A.N., Dan, M. & Smith, M.R. (1993). Propagation of Waldenström's macroglobulinemia cells in vitro and in

- severe combined immune deficient mice: utility as a preclinical drug screening model. *Blood*, Vol. 81, No. 11, (June, 1993), pp. 3034-3042, ISSN 0006-4971
- Al-Katib, A.M., Mensah-Osman, E., Aboukameel, A. & Mohammad, R. (2003). The Wayne State University Waldenstrom's Macroglobulinemia preclinical model for Waldenstrom's macroglobulinemia. *Seminars in Oncology.*, Vol. 30, No. 2, (April, 2003), pp. 313-317, ISSN 0093-7754
- Ansell, S.M., Kyle, R.A., Reeder, C.B., Fonseca, R., Mikhael, J.R., Morice, W.G., Bergsagel, P.L., Buadi, F.K., Colgan, J.P., Dingli, D., Dipenziari, A., Greipp, P.R., Habermann, T.M., Hayman, S.R., Inwards, D.J., Johnston, P.B., Kumar, S.K., Lacy, M.Q., Lust, J.A., Markovic, S.N., Micallef, I.N., Nowakowski, G.S., Porrata, L.F., Roy, V., Russell, S.J., Short, K.E., Stewart, A.K., Thompson, C.A., Witzig, T.E., Zeldenrust, S.R., Dalton, R.J., Rajkumar, S.V. & Gertz, M.A. (2010). Diagnosis and management of Waldenström macroglobulinemia: Mayo stratification of macroglobulinemia and risk-adapted therapy (mSMART) guidelines. *Mayo Clinic Proceedings.*, Vol. 85, No.9, (September, 2010), pp. 824-33, ISSN 0025-6196
- Aparicio, A., Gardner, A., Tu, Y., Savage, A., Berenson, J. & Lichtenstein, A. (1998). In vitro cytoreductive effects on multiple myeloma cells induced by bisphosphonates. *Leukemia.*, Vol. 12, No. 2, (February, 1998), pp. 220-229, ISSN 0887-6924
- Ardeshtna, K.M., Smith, P., Norton, A., Hancock, B.W., Hoskin, P.J., MacLennan, K.A., Marcus, R.E., Jelliffe, A., Vaughan, G., Hudson & Linch, D.C.; British National Lymphoma Investigation. (2003). Long-term effect of a watch and wait policy versus immediate systemic treatment for asymptomatic advanced-stage non-Hodgkin lymphoma: a randomised controlled trial. *Lancet*, Vol. 362, No. 9383, (August, 2003), pp. 516-522, ISSN 0140-6736
- Ashfaq, K., Yahaya, I., Hyde, C., Andronis, L., Barton, P., Bayliss, S. & Chen, Y.F. (2010). Clinical effectiveness and cost-effectiveness of stem cell transplantation in the management of acute leukaemia: a systematic review. *Health Technology Assessment.* Vol. 14, No. 54:iii-iv, ix-xi, pp. 1-141, ISSN 1366-5278
- Aydin, S., Grabellus, F., Eisele, L., Möllmann, M., Hanoun, M., Ebeling, P., Moritz, T., Carpinteiro, A., Nüchel, H., Sak, A., Göthert, J.R., Dührsen, U. & Dürig, J. (2011). Investigating the role of CD38 and functionally related molecular risk factors in the CLL NOD/SCID xenograft model. *European Journal of Haematology.*, Vol. 87, No. 1, (July, 2011), pp. 10-19:10-9. doi: 10.1111/j.1600-0609.2011.01626.x, ISSN 0902-4441
- Bailey, H. D., Armstrong, B. K., De Klerk, N. H., Fritschi, L., Attia, J., Lockwood, L., Milne, E.; Aus-ALL Consortium. (2010). Exposure to diagnostic radiological procedures and the risk of childhood acute lymphoblastic leukemia. *Cancer Epidemiology Biomarkers and Prevention*, Vol. 19, No. 11, (November, 2010), pp. 2897-2909, ISSN 1055-9965
- Barlogie, B., Shaughnessy, J., Munshi, N. & Epstein, J. (2001). Plasma cell myeloma. In: *Williams Hematology*, E. Beutler, M. Lichtman, B. Coller, T. Kipps, & U. Seligsohn, (Eds.; ed 6). New York: McGraw-Hill; pp. 1279-1304, ISBN 0-07-116293-3
- Barth, R.N., Nam, A.J., Stanwix, M.G., Kukuruga, D., Drachenberg, C.I., Bluebond-Langner, R., Hui-Chou, H., Shipley, S.T., Bartlett, S.T. & Rodriguez, E.D. (2009). Prolonged survival of composite facial allografts in non-human primates associated with posttransplant lymphoproliferative disorder. *Transplantation.*, Vol. 88, No. 11, (December, 2009), pp. 1242-1250, ISSN 0041-1337

- Benjamin, M., Reddy, S. & Brawley, O.W. (2003). Myeloma and race: a review of the literature. *Cancer Metastasis Reviews*, Vol. 22, No. 1, (March, 2003), pp. 87-93, ISSN 1573-7233
- Bertilaccio, M.T., Scielzo, C., Simonetti, G., Ponzoni, M., Apollonio, B., Fazi, C., Scarfò, L., Rocchi, M., Muzio, M., Caligaris-Cappio, F. & Ghia, P. (2010). A novel Rag2-/-gammac-/-xenograft model of human CLL. *Blood*, Vol. 115, No. 8, (February, 2010), pp. 1605-1609, ISSN 0006-4971
- Bhatia, S., Ramsay, N.K., Steinbuch, M., Dusenbery, K.E., Shapiro, R.S., Weisdorf, D.J., Robison, L.L., Miller, J.S. & Neglia, J.P. (1996). Malignant neoplasms following bone marrow transplantation. *Blood*, Vol. 87, No. 9, (May, 1996), pp. 3633-3639, ISSN 0006-4971
- Billadeau, D., Ahmann, G., Greipp, P. & Van Ness, B. (1993). The bone marrow of multiple myeloma patients contains B cell populations at different stages of differentiation that are clonally related to the malignant plasma cell. *Journal of Experimental Medicine*, Vol. 178, No. 3, (September, 1993), pp. 1023-1031, ISSN 0022-1007
- Blattner, W.A., Garber, J.E., Mann, D.L., McKeen, E.A., Henson, R., McGuire, D.B., Fisher, W.B., Bauman, A.W., Goldin, L.R. & Fraumeni, Jr, J.F. (1980). Waldenström's macroglobulinemia and autoimmune disease in a family. *Annals of Internal Medicine*, Vol. 93, No. 6, (December, 1980), pp. 830-832, ISSN 0003-4819
- Boulos, N., Mulder, H.L., Calabrese, C.R., Morrison, J.B., Rehg, J.E., Relling, M.V., Sherr, C.J., & Williams, R.T. (2011). Chemotherapeutic agents circumvent emergence of dasatinib-resistant BCR-ABL kinase mutations in a precise mouse model of Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*, Vol. 117, No. 13, (March, 2011), pp. 3585-3595, ISSN 0006-4971
- Boynton, E., Aubin, J., Gross, A., Hozumi, N. & Sandhu, J. (1996). Human osteoblasts survive and deposit new bone when human bone is implanted in SCID mouse. *Bone*, Vol. 18, No. 4, (April, 1996), pp. 321-326, ISSN 8756-3282
- Brown, A.K., Elves, M.W., Gunson, H.H. & Pell-Ilderton, R. (1967). Waldenström's macroglobulinaemia. A family study. *Acta Haematologica*, Vol. 38, No. 3, pp. 184-192, ISSN 0001-5792
- Caligaris-Cappio, F. & Ghia, P. (2007). The normal counterpart to the chronic lymphocytic leukemia B cell. *Best Practice & Research: Clinical Haematology*, Vol. 20, No. 3 (September, 2007), pp. 385-397, ISSN 1521-6926
- Cancer Research, U.K. (2010). Latest UK cancer incidence and mortality summary-numbers. Available from:  
<http://info.cancerresearchuk.org/cancerstats/incidence/index.htm>
- Carpenter, P.A., Appelbaum, F.R., Corey, L., Deeg, H.J., Doney, K., Gooley, T., Krueger, J., Martin, P., Pavlovic, S., Sanders, J., Slattery, J., Levitt, D., Storb, R., Woolfrey, A. & Anasetti, C. (2002). A humanized non-FcR-binding anti-CD3 antibody, visilizumab, for treatment of steroid-refractory acute graft-versus-host disease. *Blood*, Vol. 99, No. 8, (April, 2002), pp. 2712-2719, ISSN 0006-4971
- Chen, S.S., Sherman, M.H., Hertlein, E., Johnson, A.J, Teitell, M.A., Byrd, J.C. & Plass, C. (2009). Epigenetic alterations in a murine model for chronic lymphocytic leukemia. *Cell Cycle*, Vol. 8, No. 22, (November, 2009), pp. 3663-3667, ISSN 1538-4101
- Cho, P.S., Mueller, N.J., Cameron, A.M., Cina, R.A., Coburn, R.C., Hettiaratchy, S., Melendy, E., Neville, D.M. Jr, Patience, C., Fishman, J.A., Sachs, D.H. & Huang, C.A. (2004).

- Risk factors for the development of post-transplant lymphoproliferative disorder in a large animal model. *American Journal of Transplantation*, Vol. 4, No. 8, (August, 2004), pp. 1274-1282, ISSN 1600-6135
- Cohen, J.I. (1991). Epstein-Barr virus lymphoproliferative disease associated with acquired immunodeficiency. *Medicine (Baltimore)*, Vol. 70, No. 2, (March, 1991), pp. 137-160, ISSN 0025-7974
- Corradini, P., Boccadoro, M., Voena, C. & Pileri, A. (1993). Evidence for a bone marrow B cell transcribing malignant plasma cell VDJ joined to C mu sequence in immunoglobulin (IgG)- and IgA secreting multiple myelomas. *Journal of Experimental Medicine*, Vol. 178, No. 3, (September, 1993), pp. 1091-1096, ISSN 0022-1007
- Curtis, R.E., Travis, L.B., Rowlings, P.A., Socié, G., Kingma, D.W., Banks, P.M., Jaffe, E.S., Sale, G.E., Horowitz, M.M., Witherspoon, R.P., Shriner, D.A., Weisdorf, D.J., Kolb, H.J., Sullivan, K.M., Sobocinski, K.A., Gale, R.P., Hoover, R.N., Fraumeni, J.F. Jr, & Deeg, H.J. (1999). Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. *Blood*, Vol. 94, No. 7, (October, 1999), pp. 2208-2216, ISSN 0006-4971
- D'Amato, R.J., Loughnan, M.S., Flynn, E. & Folkman, J. (1994). Thalidomide is an inhibitor of angiogenesis. *Proceedings of the National Academy of Sciences U S A*, Vol. 91, No. 9, (April, 1994), pp. 4082-4085, ISSN 0027-8424
- Dang, A.M., Phillips, J.A., Lin, T. & Raveche, E.S. (1996). Altered CD45 expression in malignant B-1 cells. *Cellular Immunology*, Vol. 169, No. 2, (May, 1996), pp. 196-207, ISSN 0008-8749
- Deeg, H.J. & Socié, G. (1998). Malignancies after hematopoietic stem cell transplantation: Many questions, some answers. *Blood*, Vol. 91, No. 6, (March, 1998), pp. 1833-1844, ISSN 0006-4971
- Dimopoulos, M.A., Galani, E. & Matsouka, C. (1999). Waldenström's macroglobulinemia. *Hematology/Oncology Clinics of North America*, Vol. 13, No. 6, (December, 1999), pp. 1351-1366, ISSN 0889-8588
- Dimopoulos, M.A., Panayiotidis, P., Moulopoulos, L.A., Sfikakis, P. & Dalakas, M. (2000). Waldenström's macroglobulinemia: clinical features, complications, and management. *Journal of Clinical Oncology*, Vol. 18, No. 1, (January, 2000), pp. 214-226, ISSN 0732-183X
- Dimopoulos, M.A., Kyle, R.A., Anagnostopoulos, A. & Treon, S.P. (2005). Diagnosis and management of Waldenström's macroglobulinemia. *Journal of Clinical Oncology*, Vol. 23, No. 7, (March, 2005), pp. 1564-1577, ISSN 0732-183X
- Dimopoulos, M.A., Gertz, M.A., Kastiris, E., Garcia-Sanz, R., Kimby, E.K., Leblond, V., Femand, J.P., Merlini, G., Morel, P., Morra, E., Ocio, E.M., Owen, R., Ghobrial, I.M., Seymour, J., Kyle, R.A. & Treon, S.P. (2009). Update on treatment recommendations from the Fourth International Workshop on Waldenström's Macroglobulinemia. *Journal of Clinical Oncology*, Vol. 27, No. 1, (January, 2009), pp. 120-126, ISSN 0732-183X
- Ditzel Santos, D., Ho, A.W., Tournilhac, O., Hatjiharissi, E., Leleu, X., Xu, L., Tassone, P., Neri, P., Hunter, Z.R., Chemaly, M.A., Branagan, A.R., Manning, R.J., Patterson, C.J., Moreau, A.S., Ciccarelli, B., Adamia, S., Kriangkum, J., Kutok, J.L., Tai, Y.T., Zhang, J., Pilarski, L.M., Anderson, K.C., Munshi, N., Treon, S.P. (2007).

- Establishment of BCWM.1 cell line for Waldenström's macroglobulinemia with productive in vivo engraftment in SCID-hu mice. *Experimental Hematology*, Vol. 35, No. 9, (September, 2007), pp. 1366-1375, ISSN 0301-472X
- Dohner, H., Stilgenbauer, S., Benner, A., Leupolt, E., Kröber, A., Bullinger, L., Döhner, K., Bentz, M. & Lichter, P. (2000). Genomic aberrations and survival in chronic lymphocytic leukemia. *New England Journal of Medicine*, Vol. 343, No. 26, (December, 2000), pp. 1910-1916, ISSN 0028-4793
- Dor, F.J., Doucette, K.E., Mueller, N.J., Wilkinson, R.A., Bajwa, J.A., McMorrow, I.M., Tseng, Y.L., Kuwaki, K., Houser, S.L., Fishman, J.A., Cooper, D.K., Huang, C.A. (2004). Posttransplant lymphoproliferative disease after allogeneic transplantation of the spleen in miniature swine. *Transplantation*, Vol. 78, No. 2, (July, 2004), pp: 286-291, ISSN 0041-1337
- Dores, G.M., Anderson, W.F., Curtis, R.E., Landgren, O., Ostroumova, E., Bluhm, E.C., Rabkin, C.S., Devesa, S.S. & Linet, M.S. (2007). Chronic lymphocytic leukaemia and small lymphocytic lymphoma: Overview of the descriptive epidemiology. *British Journal of Haematology*, Vol.139, No. 5, (December, 2007), pp. 809-819, ISSN 0007-1048
- de Jong, D. & Janz, S. (2010). Anaplastic plasmacytoma of mouse--establishing parallels between subtypes of mouse and human plasma cell neoplasia. *Journal of Pathology*, Vol. 221, No. 3, (July, 2010), pp. 242-247, ISSN 1096-9896
- Doucette, K., Dor, F.J., Wilkinson R.A., Martin, S.I., Huang, C.A., Cooper, D.K., Sachs, D.H. & Fishman, J.A. (2007). Gene expression of porcine lymphotropic herpesvirus-1 in miniature Swine with posttransplant lymphoproliferative disorder. *Transplantation*, Vol. 83, No. 1, (January, 2007), pp. 87-90, ISSN 0041-1337
- Drexler, H.G. & MacLeod, R.A. (2008). Malignant hematopoietic cell lines: in vitro models for the study of Waldenström's macroglobulinemia. *Leukemia Research*, Vol. 32, No. 11, (November, 2008), pp. 1669-1673, ISSN 0887-6924
- Eden, T. (2010). Aetiology of childhood leukaemia, *Cancer Treatment Reviews*, Vol. 36, No. 4, (June, 2010), pp. 286-297, ISSN 0305-7372
- Elves, M.W. & Brown, A.K. (1968). Cytogenetic studies in a family with Waldenström's macroglobulinaemia. *Journal of Medical Genetics*, Vol. 5, No. 2, (June, 1968), pp. 118-122, ISSN 0022-2593
- Engholm, G., Ferlay, J., Christensen, N, Bray, F., Gjerstorff, M.L., Klint, A., Kotlum, J.E., Olafsdottir, E., Pukkala, E. & Storm, H.H. (2010). NORDCAN – Cancer Incidence, Mortality, Prevalence and Prediction in the Nordic Countries, version 3.6. Association of the Nordic Cancer Registries. Danish Cancer Society. Available from: <http://www.ancr.nu>
- Feo-Zuppari, F.J., Taylor, C.W., Iwato, K., Lopez, M.H., Grogan, T.M., Odeleye, A., Hersh, E.M. & Salmon, S.E. (1992). Long-term engraftment of fresh human myeloma cells in SCID mice. *Blood*, Vol. 80, No. 11, (December, 1992), pp. 2843-2850, ISSN 0006-4971
- Ferlay, J., Autier, P., Boniol, M., Heanue, M., Colombet, M. & Boyle, P. (2007). Estimates of the cancer incidence and mortality in Europe in 2006. *Annals in Oncology*, Vol. 18, No. 3, (March, 2007), pp. 581-592, ISSN 0923-7534
- Fisher, R.I., LeBlanc, M., Press, O.W., Maloney, D.G., Unger, J.M. & Miller, T.P. (2005). New treatment options have changed the survival of patients with follicular lymphoma.

- Journal of Clinical Oncology*, Vol. 23, No. 33 (November, 2005), pp. 8447-8452, ISSN 0732-183X
- Fonseca, R. & Hayman, S. (2007). Waldenström macroglobulinaemia. *British Journal of Haematology*, Vol. 138, No. 6 (September, 2007), pp. 700-720, ISSN 0007-1048
- Fonseca, R., Barlogie, B., Bataille, R., Bastard, C., Bergsagel, P.L., Chesi, M., Davies, F.E., Drach, J., Greipp, P.R., Kirsch, I.R., Kuehl, W.M., Hernandez, J.M., Minvielle, S., Pilarski, L.M., Shaughnessy, J.D. Jr, Stewart A.K. & Avet-Loiseau, H. (2004). Genetics and cytogenetics of multiple myeloma: a workshop report. *Cancer Research*, Vol. 64, No. 4, (February, 2004), pp.1546-1558, ISSN 0008-5472
- Fowler, J.A., Mundy, G.R., Lwin, S.T., Lynch, C.C. & Edwards, C.M. (2009). A murine model of myeloma that allows genetic manipulation of the host microenvironment. *Disease Models & Mechanisms*, (Vol. 2), No. 11-12, (Nov-Dec, 2009), pp. 604-611, ISSN 1754-8403
- Friedberg, J.W., Taylor, M.D., Cerhan, J.R., Flowers, C.R., Dillon, H., Farber, C.M., Rogers, E.S., Hainsworth, J.D., Wong, E.K., Vose, J.M., Zelenetz, A.D. & Link, B.K. (2009). Follicular lymphoma in the United States: first report of the national LymphoCare study. *Journal of Clinical Oncology*, Vol. 27, No. 8, (March, 2009), pp. 1202-1208, ISSN 0732-183X
- Garrett, I.R., Dallas, S., Radl, J., & Mundy, G. R. (1997). A murine model of human myeloma bone disease. *Bone*, Vol. 20, No. 6, (June, 1997), pp. 515-520, ISSN 8756-3282
- Garnier-Hausser, A., Patel, R., Baldwin, A.S., May, M.J. & Mason, N.J. (2011). NEMO-binding domain peptide inhibits constitutive NF- $\kappa$ B activity and reduces tumor burden in a canine model of relapsed, refractory diffuse large B-cell lymphoma. *Clinical Cancer Research*, Vol. 17, No. 14, (July, 2011), pp. 4661-4671, ISSN 1078-0432
- Gertz, M.A., Anagnostopoulos, A., Anderson, K., Branagan, A.R., Coleman, M., Frankel, S.R., Giralt, S., Levine, T., Munshi, N., Pestronk, A., Rajkumar, V. & Treon, S.P. (2003). Treatment recommendations in Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. *Seminars in Oncology*, Vol. 30, No. 2, (April, 2003), pp. 121-126, ISSN 0093-7754
- Getaz, E.P. & Staples, W.G. (1977). Familial Waldenström's macroglobulinaemia: a case report. *South African Medical Journal*, Vol. 51, No. 24, (June, 1977), pp. 891-892, ISSN 0038-2469
- Ghia, P., Scielzo, C., Frenquelli, M., Muzio, M., & Caligaris-Cappio, F. (2007). From normal to clonal B cells: Chronic lymphocytic leukemia (CLL) at the crossroad between neoplasia and autoimmunity. *Autoimmunity Reviews*, Vol. 7, No. 2, (December, 2007), pp. 127-131, ISSN 1568-9972
- Goedert, J.J., Cote, T.R., Virgo, P., Scoppa, S.M., Kingma, D.W., Gail, M.H., Jaffe, E.S. & Biggar, R.J. (1998). Spectrum of AIDS-associated malignant disorders. *Lancet*, Vol. 351, No. 9119, (June, 1998), pp. 1833-1839, ISSN 0140-6736
- Greaves, M. (2002). Science, medicine, and the future: childhood leukaemia. *British Medical Journal*, Vol. 324, No. 7332, pp. 283-287, ISSN 0959-8138
- Groves, F.D., Travis, L.B., Devesa, S.S., Ries, L.A. & Fraumeni, J.F. Jr. (1998). Waldenström's macroglobulinemia: incidence patterns in the United States, 1988-1994. *Cancer*, Vol. 82, No. 6, (March, 1998), pp. 1078-1081, ISSN 0008-543X

- Gurney, J.G., Davis, S., Severson, R.K., Fang, J.Y., Ross, J.A. & Robison, L.L. (1996). Trends in cancer incidence among children in the U.S. *Cancer*, Vol. 78, No. 3, (August, 1996), pp. 532-541, ISSN 0008-543X
- Gustafsson, A., Levitsky, V., Zou, J.Z., Frisan, T., Dalianis, T., Ljungman, P., Ringden, O., Winiarski, J., Ernberg, I., & Masucci, M.G. (2000). Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T cells. *Blood*, Vol. 95, No. 3, (February, 2000), pp. 807-814, ISSN 0006-4971
- Hallek, M., Langenmayer, I., Nerl, C., Knauf, W., Dietzfelbinger, H., Adorf, D., Ostwald, M., Busch, R., Kuhn-Hallek, I., Thiel, E. & Emmerich, B. (1999). Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early, nonmolding chronic lymphocytic leukemia. *Blood*, Vol. 93, No. 5, (March, 1999), pp.1732-1737, ISSN 0006-4971
- Harousseau, J.L., Shaughnessy, J.Jr & Richardson, P. (2004). Multiple myeloma. *Hematology, American Society of Hematology Educational Program*, pp. 237-256, ISSN 1520-4391
- Harris, N.L., Jaffe, E.S., Stein, H., Banks, P.M., Chan, J.K., Cleary, M.L., Delsol, G., De Wolf-Peeters, C., Falini, B. & Gatter, K.C. (1994). A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group [see comments; Review; 296 refs]. *Blood*, Vol. 84, No. 5, (September, 1994), pp. 1361-1392, ISSN 0006-4971
- Herrinton, L.J. & Weiss, N.S. (1993). Incidence of Waldenström's macroglobulinemia. *Blood*, Vol. 82, No. 10, (November, 1993), pp. 3148-3150, ISSN 0006-4971
- Heslop, H.E., Ng, C.Y.C., Li, C., Smith, C.A., Loftin, S.K., Krance, R.A., Brenner, M.K. & Rooney, C.M. (1996). Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nature Medicine*, Vol. 2, No. 5, (May, 1996), pp. 551-555, ISSN 1078-8956
- Hodge, L.S., Novak, A.J., Grote, D.M., Braggio, E., Ketterling, R.P., Manske, M.K., Price Troska, T.L., Ziesmer, S.C., Fonseca, R., Witzig, T.E., Morice, W.G., Gertz, M.A. & Ansell, S.M. (2011). Establishment and characterization of a novel Waldenström macroglobulinemia cell line, MWCL-1. *Blood*, Vol. 117, No. 19, (May, 2011), e190-197, ISSN 0006-4971
- Hofbauer, J.P., Heyder, C., Denk, U., Kocher, T., Holler, C., Trapin, D., Asslaber, D., Tinhofer, I., Greil, R. & Egle, A. (2011). Development of CLL in the TCL1 transgenic mouse model is associated with severe skewing of the T-cell compartment homologous to human CLL. *Leukemia*, Vol. 25, No. 9, (September, 2011):1452-1458. doi: 10.1038/leu.2011.111. Epub 2011 May 24, ISSN 0887-6924
- Hoover, R.N. (1992). Lymphoma risks in populations with altered immunity – A search for mechanism. *Cancer Research*, Vol. 52, No. suppl. 19, (October, 1992), pp. 5477s-5478s, ISSN 0008-5472
- Horner, M.J., Ries, L.A.G., Krapcho, M., Neyman, N., Aminou, R., Howlader, N., Altekruse, S.F., Feuer, E. J., Huang, L., Mariotto, A., Miller, B. A., Lewis, D.R., Eisner, M.P., Stinchcomb, D.G. & Edwards, B.K. (Ed.); (2009). In: *SEER Cancer Statistics Review 1975–2006*. Bethesda, MD: National Cancer Institute; Available from [http://seer.cancer.gov/csr/1975\\_2006/](http://seer.cancer.gov/csr/1975_2006/) (based on November 2008 SEER data submission, posted to the SEER web site, 2009)

- Horning, S.J. (1993). Natural history of and therapy for the indolent non-Hodgkin's lymphomas. *Seminars in Oncology*, Vol. 20, No. 5, Suppl. 5, (October, 1993), pp. 75–88, ISSN 0093-7754
- Howlader, N., Noone, A.M., Krapcho, M., Neyman, N., Aminou, R., Waldron, W., Altekruse, S.F., Kosary, C.L., Ruhl, J., Tatalovich, Z., Cho, H., Mariotto, A., Eisner, M.P., Lewis, D.R., Chen, H.S., Feuer, E.J., Cronin, K.A. & Edwards, B.K. (2010). In: *SEER Cancer Statistics Review, 1975–2008*, National Cancer Institute, Bethesda, MD. Available from: [http://seer.cancer.gov/csr/1975\\_2008/](http://seer.cancer.gov/csr/1975_2008/)
- Huang, Y.W., Richardson, J.A., Tong, A.W., Zhang, B.Q., Stone, M.J. & Vitetta, E.S. (1993). Disseminated growth of a human multiple myeloma cell line in mice with severe combined immunodeficiency disease. *Cancer Research*, Vol. 53, No. 6, (March, 1993), pp. 1392-1396, ISSN 0008-5472
- Jemal, A., Siegel, R., Xu, J. & Ward, E. (2010). Cancer statistics, 2010. *CA: A Cancer Journal for Clinicians*, Vol. 60, No. 5, pp. 277-300, ISSN 0007-9235
- Johnson, P.W., Rohatiner, A.Z., Whelan, J.S., Price, C.G., Love, S., Lim, J., Matthews, J., Norton, A.J., Amess, J.A. & Lister, T.A. (1995). Patterns of survival in patients with recurrent follicular lymphoma: a 20-year study from a single center. *Journal of Clinical Oncology*, Vol. 13, No. 1, (January, 1995), pp. 140–147. ISSN 0732-183X
- Kamel-Reid, S., Letarte, M., Sirard, C., Doedens, M., Grunberger, T., Fulop, G., Freedman, M.H., Phillips, R.A. & Dick, J.E. (1989). A model of human acute lymphoblastic leukemia in immune-deficient SCID mice. *Science*, Vol. 246, No. 4937, (December, 1989), pp. 1597-1600, ISSN 0036-8075
- Kinlen, L.J. (1996). Immunologic factors, including AIDS, In: *Cancer Epidemiology and Prevention*, D. Schottenfeld & J.F. Fraumeni Jr, (Eds): (Ed 2), pp. 532-545, ISBN 978-0-19-514961-6, Oxford University Press, New York, NY, USA
- Kuehl, W.M. & Bergsagel, P.L. (2002). Multiple myeloma: evolving genetic events and host interactions. *Nature Reviews Cancer*, Vol. 2, No. 3, (March, 2002), pp. 175-187, ISSN 1474-175X
- Kuehnle, I., Huls, M.H., Liu, Z., Semmelmann, M., Krance, R.A., Brenner, M.K., Rooney, C.M. & Heslop, H.E. (2000). CD20 monoclonal antibody (rituximab) for therapy of Epstein-Barr virus lymphoma after hemopoietic stem-cell transplantation. *Blood*, Vol. 95, No. 4, (February, 2000), pp. 1502-1505, ISSN 0006-4971
- Kyle, R.A., Therneau, T.M., Rajkumar, S.V., Offord, J.R., Larson, D.R., Plevak, M.F. & Melton, III, L.J. (2002). A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *New England Journal of Medicine*, Vol. 346, No. 8, (February, 2002), pp. 564–569, ISSN 0028-4793
- Kyle, R.A. & Rajkumar, S.V. (2004). Multiple myeloma. *New England Journal of Medicine*, Vol. 351, No. 18, (October, 2004), pp. 1860–1873, ISSN 0028-4793
- Lanasa, M.C., Allgood, S.D., Volkheimer, A.D., Gockerman, J.P., Whitesides, J.F., Goodman, B.K., Moore, J.O., Weinberg, J.B. & Levesque, M.C. (2010). Single-cell analysis reveals oligoclonality among 'low-count' monoclonal B-cell lymphocytosis. *Leukemia*, Vol. 24, No. 1, (January, 2010), pp. 133–140, ISSN 0887-6924
- Langenau, D.M., Traver, D., Ferrando, A.A., Kutok, J.L., Aster, J.C., Kanki, J.P., Lin, S., Prochownik, E., Trede, N.S., Zon, L.I. & Look, A.T. (2003). Myc-induced T cell leukemia in transgenic zebrafish. *Science*, Vol. 299, No. 5608, (February, 2003), pp. 887-890, ISSN 0036-8075

- Lichtman, M.A. & Liesveld, J.L. (2001). Acute Myelogenous Leukemia. In: *Williams Hematology*, E. Beutler, M. Lichtman, B. Collier, T. Kipps, & U. Seligsohn, (Eds.; ed 6), pp. 1047-1083, McGraw-Hill, ISBN 0-07-116293-3, New York, NY, USA
- Linnet, M.S., Humphrey, R.L., Mehl, E.S., Brown, L.M., Pottern, L.M., Bias, W.B. & McCaffrey, L. (1993). A case-control and family study of Waldenström's macroglobulinemia. *Leukemia*, Vol. 7, No. 9 (September, 1993), pp. 1363-1369, ISSN 0887-6924
- Linnet, M.S., Devesa, S.S. & Morgan, G.J. (2006). The leukemias, In: *Cancer Epidemiology and Prevention. 3rd edition*, D. Schottenfeld, & J.F. Fraumeni, (Ed.), 841-871, Oxford University Press, ISBN 978-0-19-514961-6, New York, USA
- Liu, Q., Fayad, L., Cabanillas, F., Hagemeister, F.B., Ayers, G.D., Hess, M., Romaguera, J., Rodriguez, M.A., Tsimberidou, A.M., Verstovsek, S., Younes, A., Pro, B., Lee, M.S., Ayala, A., McLaughlin, P. (2006). Improvement of Overall and Failure-Free Survival in Stage IV Follicular Lymphoma: 25 Years of Treatment Experience at The University of Texas M.D. Anderson Cancer Center. *Journal of Clinical Oncology*, Vol. 24, No. 10, (April, 2006), pp. 1582-1589, ISSN 0732-183X
- Liu, R., Zhang, L., McHale, C.M. & Hammond, S.K. (2011). Paternal smoking and risk of childhood acute lymphoblastic leukemia: systematic review and meta-analysis. *Journal of Oncology*, 2011:854584. Epub 2011 May, 29, doi:10.1155/2011/854584, ISSN 1687-8450
- Lock, R.B., Liem, N., Farnsworth, M.L., Milross, C.G., Xue, C., Tajbakhsh, M., Haber, M., Norris, M.D., Marshall, G.M. & Rice, A.M. (2002). The nonobese diabetic/severe combined immunodeficient (NOD/SCID) mouse model of childhood acute lymphoblastic leukemia reveals intrinsic differences in biologic characteristics at diagnosis and relapse. *Blood*, Vol. 99, No. 11, (June, 2002), pp. 4100-4108, ISSN 0006-4971
- Macor, P., Secco, E., Zorzet, S., Tripodo, C., Celeghini, C. & Tedesco, F. (2008). An update on the xenograft and mouse models suitable for investigating new therapeutic compounds for the treatment of B-cell malignancies. *Current Pharmaceutical Design*, Vol. 14, No. 21, pp. 2023-2039, ISSN 1381-6128
- Marcus, R., Imrie, K., Belch, A., Cunningham, D., Flores, E., Catalano, J., Solal-Celigny, P., Offner, F., Walewski, J., Raposo, J., Jack, A. & Smith P. (2005). CVP chemotherapy plus rituximab compared with CVP as firstline treatment for advanced follicular lymphoma. *Blood*, Vol. 105, No. 4, (February, 2005), pp. 1417-1423, ISSN 0006-4971
- McMaster, M.L., Giambarresi, T., Vasquez, L., Goldstein, A.M. & Tucker, M.A. (2005). Cytogenetics of familial Waldenström's macroglobulinemia: in pursuit of an understanding of genetic predisposition. *Clinical Lymphoma*, Vol. 5, No. 4, (March, 2005), pp. 230-234, ISSN 1526-9655
- McMaster, M.L., Goldin, L.R., Bai, Y., Ter-Minassian, M., Boehringer, S., Giambarresi, T.R., Vasquez, L.G. & Tucker, M.A. (2006). Genomewide linkage screen for Waldenström macroglobulinemia susceptibility loci in high-risk families. *American Journal of Human Genetics*, Vol. 79, No. 4 (October, 2006), pp. 695-701, ISSN 0002-9297
- Medyouf, H., Gao, X., Armstrong, F., Gusscott, S., Liu, Q., Gedman, A.L., Matherly, L.H., Schultz, K.R., Pflumio, F., You, M.J. & Weng, A.P. (2010). Acute T-cell leukemias remain dependent on Notch signaling despite PTEN and INK4A/ARF loss. *Blood*, Vol. 115, No. 6, (February, 2010), pp. 1175-1184, ISSN 0006-4971

- Montoto, S., López-Guillermo, A., Ferrer, A., Camós, M., Alvarez-Larrán, A., Bosch, F., Bladé, J., Cervantes, F., Esteve, J., Cobo, F., Colomer, D., Campo, E. & Montserrat, E. (2002). Survival after progression in patients with follicular lymphoma: analysis of prognostic factors. *Annals of Oncology*, Vol. 13, No. 4, pp. 523–530, ISSN 0923-7534
- Mukherjee, M., Ge, G., Zhang, N., Huang, E., Nakamura, L.V., Minor, M., Fofanov, V., Rao, P.H., Herron, A. & Pati, D. (2011). Separate loss of function cooperates with the loss of p53 in the initiation and progression of T- and B-cell lymphoma, leukemia and aneuploidy in mice. *PLoS One*, Vol. 6, No. 7:e22167. Epub 2011 Jul 25, ISSN 1932-6203
- National Cancer Institute (2009). General information about adult non-Hodgkin's lymphoma, Available from: <http://www.cancer.gov.libproxy.lib.unc.edu/cancertopics/pdq/treatment/adult-non-Hodgkins>
- Newell, K.A., Alonso, E.M., Whittington, P.F., Bruce, D.S., Millis, J.M., Piper, J.B., Woodle, E.S., Kelly, S.M., Koeppe, H., Hart, J., Rubin, C.M. & Thistlethwaite, J.R. Jr. (1996). Posttransplant lymphoproliferative disease in pediatric liver transplantation. Interplay between primary Epstein-Barr virus infection and immunosuppression. *Transplantation*, Vol. 62, No. 3, (August, 1996), pp. 370-375, ISSN 0041-1337
- Nijmeijer, B.A., Mollevanger, P., van Zelderen-Bhola, S.L., Kluin-Nelemans, H.C., Willemze, R. & Falkenburg, J.H. (2001). Monitoring of engraftment and progression of acute lymphoblastic leukemia in individual NOD/SCID mice. *Experimental Hematology*, Vol. 29, No. 3, (March, 2001), pp. 322-329, ISSN 0301-472X
- Ohyashiki, J.H., Ohyashiki, K., Iwama, H., Hayashi, S., Toyama, K. & Shay, J.W. (1997). Clinical implications of telomerase activity levels in acute leukemia. *Clinical Cancer Research*, Vol. 3, No. 4, (April, 1997), pp. 619-625, ISSN 0008-5472
- Ogmundsdottir, H.M., Sveinsdottir, S., Sigfusson, A., Skaftadottir, I., Jonasson, J.G. & Agnarsson, B.A. (1999). Enhanced B cell survival in familial macroglobulinaemia is associated with increased expression of Bcl-2. *Clinical & Experimental Immunology*, Vol. 117, No. 2, (August, 1999), pp. 252–260, ISSN 0009-9104
- Opelz, G. & Henderson, R (1993). Incidence of non-Hodgkin's lymphoma in kidney and heart transplant recipients. *Lancet*, Vol. 342, No. 8886-8887, (December, 1993), pp. 1514-1516, ISSN 0140-6736
- Orazi, A., Hromas, R.A., Neiman, R.S., Greiner, T.C., Lee, C.H., Rubin, L., Haskins, S., Heerema, N.A., Gharpure, V., Abonour, R. Srour, E.F., & Cornetta, K. (1997). Posttransplantation lymphoproliferative disorders in bone marrow transplant recipients are aggressive diseases with a high incidence of adverse histologic and immunobiologic features. *American Journal of Clinical Pathology*, Vol. 107, No. 4, (April, 1997), pp. 419-429, ISSN 0002-9173
- O'Reilly, R.J., Lacerda, J.F., Lucas, K.G., Rosenfield, N.S., Small, T.N. & Papadopoulos, E.B. (1996). Adoptive cell therapy with donor lymphocytes for EBV-associated lymphomas developing after allogeneic marrow transplants. In: *Important Advances in Oncology*, T.D. De Vita, S. Helman & S.A. Rosenberg (Eds), pp. 149-166, Lippincott-Raven, ISBN 0-7020-1546-6, Philadelphia, PA, USA
- Owen, R.G., Treon, S.P., Al-Katib, A., Fonseca, R., Greipp, P.R., McMaster, M.L., Morra, E., Pangalis, G.A., San Miguel, J.F., Branagan, A.R., & Dimopoulos, M.A. (2003). Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus

- panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. *Seminars in Oncology*, Vol. 30, No. 2, (April, 2003), pp. 110-115, ISSN 0093-7754
- Park, S.S., Kim, J.S., Tessarollo, L., Owens, J.D., Peng, L., Han, S.S., Tae, Chung, S., Torrey, T.A., Cheung, W.C., Polakiewicz, R.D., McNeil, N., Ried, T., Mushinski, J.F., Morse, H.C. 3<sup>rd</sup> & Janz, S. (2005). Insertion of c-Myc into Igh induces B-cell and plasma-cell neoplasms in mice. *Cancer Research*, Vol. 65, No. 4 (February, 2005), pp. 1306-1315, ISSN 0008-5472
- Petrelli, N.J., Winer, E.P., Brahmer, J., Dubey, S., Smith, S., Thomas, C., Vahdat, L.T., Obel, J., Vogelzang, N., Markman, M., Sweetenham, J.W., Pfister, D., Kris, M.G., Schuchter, L.M., Sawaya, R., Raghavan, D., Ganz, P.A. & Kramer, B. (2009). Clinical Cancer Advances 2009: major research advances in cancer treatment, prevention, and screening—a report from the American Society of Clinical Oncology. *Journal of Clinical Oncology*, Vol. 27, No. 35, (December, 2009), pp. 6052-6069, ISSN 0732-183X
- Phillips, J.A., Mehta, K., Fernandez, C. & Raveche, E.S. (1992). The NZB mouse as a model for chronic lymphocytic leukemia. *Cancer Research*, Vol. 52, No. 2, (January, 1992), pp. 437-443, ISSN 0008-5472
- Potter, M. (1982). Pathogenesis of plasmacytomas in mice, In: *Cancer: A Comprehensive Treatise*, F. F. Becker, (Ed.), 139, Plenum, New York, NY, USA
- Pui, C.-H. (2001). Acute Lymphoblastic Leukemia. In: *Williams Hematology*, E. Beutler, M. Lichtman, B. Coller, T. Kipps, & U. Seligsohn, (Eds.; ed 6), McGraw-Hill; pp. 1141-1161, ISBN 0-07-116293-3, New York, USA
- Pui, C. & Evans, W.E. (2006). Treatment of acute lymphoblastic leukemia. *New England Journal of Medicine*, Vol. 354, No. 2, (January, 2006), pp. 166-178, ISSN 0028-4793
- Pui, C., Campana, D., Pei, D., Bowman, W.P., Sandlund, J.T., Kaste, S.C., Ribeiro, R.C., Rubnitz, J.E., Raimondi, S.C. Onciu, M., Coustan-Smith, E., Kun, L.E., Jeha, S., Cheng, C., Howard, S.C., Simmons, V., Bayles, A., Metzger, M.L., Boyett, J.M., Leung, W., Handgretinger, R., Downing, J.R., Evans, W.E. & Relling, M.V. (2009). Treating childhood acute lymphoblastic leukemia without cranial irradiation. *New England Journal of Medicine*, Vol. 360, No. 26, (January, 2009), pp. 2730-2741, ISSN 0028-4793
- Pulte, D., Gondos, A. & Brenner, H. (2008). Ongoing improvement in outcomes for patients diagnosed as having Non-Hodgkin lymphoma from the 1990s to the early 21st century. *Archives of Internal Medicine*, Vol. 168, No. 5, (March, 2008), pp. 469-476, ISSN 0003-9926
- Radl, J., De Glopper, E.D., Schuit, H.R., & Zurcher, C. (1979). Idiopathic paraproteinemia. II. Transplantation of the paraprotein-producing clone from old to young C57BL/KaLwRij mice. *Journal of Immunology*, Vol. 122, No. 2, (February, 1979), pp. 609-613, ISSN 0022-1767
- Radl, J. (1981). Animal model of human disease. Benign monoclonal gammopathy (idiopathic paraproteinemia). *American Journal of Pathology*, Vol. 105, No. 1, (October, 1981), pp. 91-93, ISSN 0002-9440
- Radl, J., Croese, J.W., Zurcher, C. Van den Enden-Vieveen, M. H. & de Leeuw, A. M. (1988). Animal model of human disease. Multiple myeloma. *American Journal of Pathology*, Vol. 132, No. 3, (September, 1988), pp. 593-597, ISSN 0002-9440

- Radl, J. (1991). Four major mechanisms in the development of monoclonal gammopathies. Postulations and facts, *Proceedings of the Third EURAGE Symposium on Monoclonal Gammopathies: Clinical Significance and Basic Mechanisms*, pp. 5, ISBN 907-1021-14-9, Brussels, Belgium, September 18-20, 1991
- Ramachandra, S., Metcalf, R.A., Fredrickson, T., Marti, G.E. & Raveche, E. (1996). Requirement for increased IL-10 in the development of B-1 lymphoproliferative disease in a murine model of CLL. *Journal of Clinical Investigation*, Vol. 98, No. 8, (October, 1996), pp. 1788–1793, ISSN 0021-9738
- Redaelli, A., Laskin, B.L., Stephens, J.M., Botteman, M.F. & Pashos, C.L. (2004). The clinical and epidemiological burden of chronic lymphocytic leukaemia. *European Journal of Cancer Care (Engl)*, Vol.13, No. 3, (July, 2004), pp. 279–287, ISSN 0961-5423
- Renier, G., Ifrah, N., Chevaller, A., Saint-Andre, J.P., Boasson, M. & Hurez, D. (1989). Four brothers with Waldenstrom's macroglobulinemia. *Cancer*, Vol. 64, No. 7, (October, 1989), pp. 1554–1559, ISSN 0008-543X
- Rooney, C.M., Smith, C.A., Ng, C.Y.C., Loftin, S.K., Sixbey, J.W., Gan, Y., Srivastava, D.K., Bowman, L.C., Krance, R.A., Brenner, M.K. & Heslop, H.E. (1998). Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood*, Vol. 92, No. 5, (September, 1998), pp. 1549-1555, ISSN 0006-4971
- Rozman, C. & Montserrat, E. (1995). Chronic lymphocytic leukemia. *New England Journal of Medicine*, Vol. 333, No. 16, (October, 1995), pp.1052-1057, ISSN 0028 4793
- Ruiz-Arguelles, G.J., Ramirez-Cisneros, F.J., Flores-Martinez, J. & Cernuda-Graham, M.C. (2000). Waldenstrom's macroglobulinemia is infrequent in Mexican Mestizos: experience of a hematological diseases referral center. *Revista de Investigación Clínica*, Vol. 52, No. 5, (September, 2000), pp. 497–499, ISSN 0034-8376
- Salerno, E., Yuan, Y., Scaglione, B.J., Marti, G., Jankovic, A., Mazzella, F., Laurindo, M.F., Despres, D., Baskar, S., Rader, C. & Raveche, E. (2010). The New Zealand black mouse as a model for the development and progression of chronic lymphocytic leukemia. *Cytometry Part B Clinical Cytometry*, Vol. 78, No. Suppl 1, pp. S98-109, ISSN 1552-4949
- Samuels, A.L., Peeva, V.K., Papa, R.A., Firth, M.J., Francis, R.W., Beesley, A.H., Lock, R.B. & Kees U.R. (2010). Validation of a mouse xenograft model system for gene expression analysis of human acute lymphoblastic leukaemia. *BMC Genomics.*, Vol. 21, No. 11 (April, 2010), pp. 256, ISSN 1471-2164
- Santanam, U., Zanesi, N., Efanov, A., Costinean, S., Palamarchuk, A., Hagan, J.P., Volinia, S., Alder, H., Rassenti, L., Kipps, T., Croce, C.M. & Pekarsky, Y. (2010). Chronic lymphocytic leukemia modeled in mouse by targeted miR-29 expression. *Proceedings of the National Academy of Sciences U S A.*, Vol. 107, No. 27, (July, 2010), pp. 12210-12215, ISSN 0027-8424
- Santos, D.D., Ho, A.W., Tournilhac, O., Hatjiharissi, E., Leleu, X., Xu, L., Tassone, P., Neri, P., Hunter, Z.R., Chemaly, M.A., Branagan, A.R., Manning, R.J., Patterson, C.J., Moreau, A.S., Ciccarelli, B., Adamia, S., Kriangkum, J., Kutok, J.L., Tai, Y.T., Zhang, J., Pilarski, L.M., Anderson, K.C., Munshi, N. & Treon, S.P. (2007). Establishment of BCWM.1 cell line for Waldenström's macroglobulinemia with productive in vivo engraftment in SCID-hu mice. *Experimental Hematology*, Vol. 35, No. 9, (September, 2007), pp.1366-1375, ISSN 0301-472X

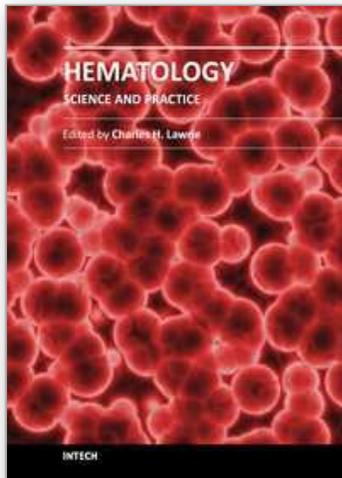
- Scaglione, B.J., Salerno, E., Balan, M., Coffman, F., Landgraf, P., Abbasi, F., Kotenko, S., Marti, G.E. & Raveche, E.S. (2007). Murine models of chronic lymphocytic leukaemia: role of microRNA-16 in the New Zealand Black mouse model. *British Journal of Haematology*, Vol. 139, No. 5, (December, 2007), pp. 645-657, ISSN 0007-1048
- Schmidtke, J., Wang, R., Wu CL, Mauiyyedi, S., Harris, N.L., Della, Pelle, P., Brousaides, N., Zagachin, L., Ferry, J.A., Wang, F., Kawai, T., Sachs, D.H., Cosimi, B.A. & Colvin, R.B. (2002). Posttransplant lymphoproliferative disorder associated with an Epstein-Barr-related virus in cynomolgus monkeys. *Transplantation*, Vol. 73, No. 9, (May, 2002), pp. 1431-1439, ISSN 0041-1337
- Seeman, E. (2008). Bone quality: the material and structural basis of bone strength. *Journal of Bone Mineral Metabolism*, Vol. 26, No. 1, (Epub January, 2008), pp. 1-8, ISSN 0914-8779
- SEER Cancer Statistics Review, 1973-1995. (1998). National Cancer Institute. Available from: [http://seer.cancer.gov/csr/1973\\_1995/overview.pdf](http://seer.cancer.gov/csr/1973_1995/overview.pdf)
- SEER (Surveillance Epidemiology and End Results); (2010), "SEER Cancer Statistics Review 1975-2007". Available from: [http://seer.cancer.gov/csr/1975\\_2007/results\\_single/sect\\_28\\_table.09.pdf](http://seer.cancer.gov/csr/1975_2007/results_single/sect_28_table.09.pdf).
- Shay, J.W., Werbin, H. & Wright, W.E. (1996). Telomeres and telomerase in human leukemias. *Leukemia*, Vol. 10, No. 8, (August, 1996), pp. 1255-1261, ISSN 0887-6924
- Shipman, C.M., Rogers, M.J., Apperley, J.F., Russell, R.G. & Croucher, P.I. (1997). Bisphosphonates induce apoptosis in human myeloma cell lines: a novel anti-tumour activity. *British Journal of Haematology*, Vol. 98, No. 3, (September, 1997), pp. 665-672, ISSN 0361-8609
- Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., Munshi, N., Anaissie, E., Wilson, C., Dhodapkar, M., Zeddis, J. & Barlogie, B. (1999). Antitumor activity of thalidomide in refractory multiple myeloma. *New England Journal of Medicine*, Vol. 341(21), (November, 1999), pp.1565-1571, ISSN 0028-4793
- Sjak-Shie, N. N., Tsingotjidou, A. S., Zhang, K., Vescio, R. A., Said, J. W., Lieberman, J. R. & Berenson, J. R. (1999). Development of a SCID-hu animal model that more closely resembles human multiple myeloma. *Blood*, Vol. 94, No. 10, pp. 2447 Part 1 Supp., 1999, ISSN 0006-4971
- Smith, A.C., Raimondi, A.R., Salthouse, C.D., Ignatius, M.S., Blackburn, J.S., Mizgirev, I.V., Storer, N.Y. de Jong, J.L., Chen, A.T., Zhou, Y., Revskoy, S., Zon, L.I., & Langenau, D.M. (2010). High-throughput cell transplantation establishes that tumor-initiating cells are abundant in zebrafish T-cell acute lymphoblastic leukemia. *Blood*, Vol. 115, No. 16, (April, 2010), pp. 3296-3303, ISSN 0006-4971
- Smith, M.A., Gloeckler Ries, L.A., Gurney, J.G. & Ross, J.A. (2010). "Leukemia. SEER Pediatric Monograph". Available from: <http://seer.cancer.gov/publications/childhood/leukemia.pdf>
- Stehbens, J.A., Kaleita, T.A., Noll, R.B., MacLean, W.E. Jr, O'Brien, R.T., Waskerwitz, M.J. & Hammond, G.D. (1991). CNS prophylaxis of childhood leukemia: what are the long-term neurological, neuropsychological, and behavioral effects? *Neuropsychology Review*, Vol. 2, No. 2, (June, 1991), pp. 147-177, ISSN 1040-7308
- Suljagic, M., Longo, P.G., Bennardo, S., Perlas, E., Leone, G., Laurenti, L. & Efremov, D.G. (2010). The Syk inhibitor fostamatinib disodium (R788) inhibits tumor growth in

- the E $\mu$ - TCL1 transgenic mouse model of CLL by blocking antigen-dependent B-cell receptor signaling. *Blood.*, Vol. 116, No. 23, (December, 2010), pp. 4894-4905, ISSN 0006-4971
- Swinnen, L.J., Costanzo-Nordin, M.R., Fisher, S.G., O'Sullivan, E.J., Johnson, M.R., Heroux, A.L., Dizikes, G.J., Pifarre, R. & Fisher, R.I. (1990). Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. *New England Journal of Medicine*, Vol. 323, No. 25, (December, 1990), pp. 1723-1728, ISSN 0028-4793
- Szcepek, A.J., Seeberger, K., Wizniak, J., Mant, M.J., Belch, A.R. & Pilarski, L.M. (1998). A high frequency of circulating B cells share clonotypic Ig heavy-chain VDJ rearrangements with autologous bone marrow plasma cells in multiple myeloma, as measured by single-cell and in situ reverse transcriptase-polymerase chain reaction. *Blood.*, Vol. 92, No. 8, (October, 1998), pp. 2844-2855, ISSN 0006-4971
- Tang, Y., He, R., Zhang, Y., Liu, F., Cheng, A., Wu, Y. & Gan, R. (2011). Human-derived IgG level as an indicator for EBV-associated lymphoma model in Hu-PBL/SCID chimeras. *Virology Journal*, Vol. 8, (May, 2011), pp. 213, ISSN 1743-422X
- Tassone, P., Neri, P., Kutok, J.L., Tournilhac, O., Santos, D.D., Hatjiharissi, E., Munshi, V., Venuta, S., Anderson, K.C., Treon, S.P. & Munshi, N.C. (2005). A SCID-hu in vivo model of human Waldenström macroglobulinemia. *Blood.*, Vol. 106, No. 4, (August, 2005), pp. 1341-1345, ISSN 0006-4971
- The Non-Hodgkin's Lymphoma Classification Project (1997). A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. *Blood*, Vol. 89, No. 11, (June, 1997), pp. 3909-3918, ISSN 0006-4971
- Theofilopoulos, A.N. (1996). Genetics of systemic autoimmunity. *Journal of Autoimmunity*, Vol. 9, No. 2, (April, 1996), pp. 207-210, ISSN 0896-8411
- Treon, S.P., Branagan, A.R., Hunter, Z., Santos, D., Tournilhac, O. & Anderson, K.C. (2004). Paradoxical increases in serum IgM and viscosity levels following rituximab in Waldenström's macroglobulinemia. *Annals in Oncology*, Vol. 15, No. 10, (October, 2004), pp. 1481-1483, ISSN 0923-7534
- Treon, S.P., Gertz, M.A., Dimopoulos, M., Anagnostopoulos, A., Blade, J., Branagan, A.R., Garcia-Sanz, R., Johnson, S., Kimby, E., Leblond, V., Femand, J.P., Maloney, D.G., Merlini, G., Morel, P., Morra, E., Nichols, G., Ocio, E.M., Owen, R. & Stone, M.J. (2006). Update on treatment recommendations from the Third International Workshop on Waldenström's Macroglobulinemia. *Blood*, Vol. 107, No. 9, (May, 2006), pp. 3442-3446, ISSN 0006-4971.
- Tsingotjidou, A.S., Zotalis, G., Jackson, K.R., Sawyers, C., Puzas, J.E., Hicks, D.G., Reiter, R. & Lieberman, J.R. (2001). Development of an animal model for prostate cancer cell metastasis to adult human bone. *Anticancer Research*, Vol. 21, No. 2A, (March-April, 2001), pp. 971-978, ISSN 0250-7005
- Tsingotjidou, A.S., Emmanouilides, C.E., Siotou, E., Poutahidis, T., Xagorari, A., Loukopoulos, P., Sotiropoulos, D., Bekiari, C., Doulberis, M., Givissis, P., Fassas, A. & Anagnostopoulos, A. (2009). Establishment of an animal model for Waldenström's macroglobulinemia. *Experimental Hematology*, Vol. 37, No.4, (April, 2009), pp. 469-476, ISSN 0301-472X
- U.S. Cancer Statistics Working group. Statistics Working Group (2009). United States Cancer Statistics: 1999-2005 Incidence and Mortality Web-based Report. Atlanta: U.S.

- Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute. Available from:  
[www.cdc.gov/uscs](http://www.cdc.gov/uscs)
- van Esser, J.W., Niesters, H.G., van der Holt, B., Meijer, E., Osterhaus, A.D., Gratama, J.W., Verdonck, L.F., Löwenberg, B. & Cornelissen, J.J. (2002). Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. *Blood*, Vol. 99, No. 12, (June, 2002), pp. 4364-4369, ISSN 0006-4971
- Vijay, A. & Gertz, M.A. (2007). Waldenström macroglobulinemia. *Blood*, Vol. 109, No. 12, (June, 2007), pp. 5096-5103, ISSN 0006-4971
- Virgilio, L., Lazzeri, C., Bichi, R., Nibu, K., Narducci, M.G., Russo G., Rothstein, J.L. & Croce C. M. (1998). Deregulated expression of TCL1 causes T cell leukemia in mice. *Proceedings of the National Academy of Sciences USA*, Vol. 95, No. 7, (March, 1998), pp. 3885-3889, ISSN 0027-8424
- Waldenström, J. (1944). Incipient myelomatosis or 'essential' hyperglobulinemia with fibrinogenopenia: a new syndrome? *Acta Medica Scandinavica*, Vol. 117, pp. 216-222, ISSN 0001-6101
- Watson, L., Wyld, P. & Catovsky D. (2008). Disease burden of chronic lymphocytic leukaemia within the European Union. *European Journal of Haematology*, Vol. 81, No. 4, (December 2008), pp. 253-258, ISSN 0902-4441
- World Health Organization (2010), "Disease and injury regional estimates for 2004". Available from:  
[http://www.who.int/healthinfo/global\\_burden\\_disease/estimates\\_regional/en/index.html](http://www.who.int/healthinfo/global_burden_disease/estimates_regional/en/index.html)
- Witherspoon, R.P., Fisher, L.D., Schoch, G., Martin, P., Sullivan, K.M., Sanders, J., Deeg, H.J., Doney, K., Thomas, D., Storb, R. & Thomas, E.D. (1989). Secondary cancers after bone marrow transplantation for leukemia or aplastic anemia. *New England Journal of Medicine*, Vol. 321, No. 12, (September, 1989), pp. 784-789, ISSN 0028-4793
- Yaccoby, S., Barlogie, B. & Epstein, J. (1998). Primary myeloma cells growing in SCID-hu mice: a model for studying the biology and treatment of myeloma and its manifestations. *Blood*, Vol. 92, No. 8, (October, 1998), pp. 2908-2913, ISSN 0006-4971
- Yaccoby, S. & Epstein, J. (1999). The proliferative potential of myeloma plasma cells manifest in the SCID-hu host. *Blood*, Vol. 94, No. 10, (November, 1999), pp. 3576-3582, ISSN 0006-4971
- Yaccoby, S, Pearse, R.N., Johnson, C.L., Barlogie, B., Choi, Y. & Epstein, J. (2002). Myeloma interacts with the bone marrow microenvironment to induce osteoclastogenesis and is dependent on osteoclast activity. *British Journal of Haematology*, Vol. 116, No. 2, (February, 2002), pp. 278-290, ISSN 0007-1048
- Yaccoby, S, Wezeman, M.J., Zangari, M., Walker, R., Cottler-Fox, M., Gaddy, D., Ling, W., Saha, R., Barlogie, B., Tricot, G., & Epstein, J. (2006). Inhibitory effects of osteoblasts and increased bone formation on myeloma in novel culture systems and a myelomatous mouse model. *Haematologica*, Vol. 91, No. 2, (February, 2006), pp. 192-199, ISSN 0390-6078
- Yaccoby, S, Ling, W., Zhan, F., Walker, R., Barlogie, B., Shaughnessy, J.D. Jr. (2007). Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption

- and multiple myeloma growth in vivo. *Blood.*, Vol. 109, No. 5, (March 2007), pp: 2106-2111, ISSN 0006-4971
- Yata, K., & Yaccoby, S. (2004). The SCID-rab model: a novel in vivo system for primary human myeloma demonstrating growth of CD138-expressing malignant cells. *Leukemia*, Vol.18, No. 11, (November 2004), pp.1891-1897, ISSN 0887-6924.
- Yonou, H., Yokose, T., Kamijo, T., Kanomata, N., Hasebe, T., Nagai, K., Hatano, T., Ogawa, Y. & Ochiai, A. (2001). Establishment of a novel species- and tissue-specific metastasis model of human prostate cancer in humanized non-obese diabetic/severe combined immunodeficient mice engrafted with human adult lung and bone. *Cancer Research*, Vol. 61, No. 5, (March, 2001), pp. 2177-2282, ISSN 0008-5472
- Yu, B.H., Zhou, X.Y., Zhang, T.C., Zhang, T.M. & Shi, D.R. (2011). Establishment and characterization of a nude mice model of human diffuse large B-cell lymphoma. *Chinese Journal of Pathology (Zhonghua Bing Li Xue Za Zhi.)*, Vol. 40, No. 4, (April, 2011), pp. 246-250, ISSN 0529-5807
- Yun, J.P., Behan, J.W., Heisterkamp, N., Butturini, A., Klemm, L., Ji, L., Groffen, J., Müschen, M. & Mittelman S.D. (2010). Diet-induced obesity accelerates acute lymphoblastic leukemia progression in two murine models. *Cancer Prevention Research (Philadelphia, PA)*, Vol. 3, No. 10, (October, 2010), pp. 1259-1264, ISSN 1940-6215
- Zhang, J., Xiao, Y., Guo, Y., Breslin, P., Zhang, S., Wei, W., Zhang, Z. & Zhang, J. (2011). Differential requirements for c-Myc in chronic hematopoietic hyperplasia and acute hematopoietic malignancies in Pten-null mice. *Leukemia*, Sep. 16. doi: 10.1038/leu.2011.220, ISSN 0887-692

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## **Hematology - Science and Practice**

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Hematology encompasses the physiology and pathology of blood and of the blood-forming organs. In common with other areas of medicine, the pace of change in hematology has been breathtaking over recent years. There are now many treatment options available to the modern hematologist and, happily, a greatly improved outlook for the vast majority of patients with blood disorders and malignancies. Improvements in the clinic reflect, and in many respects are driven by, advances in our scientific understanding of hematological processes under both normal and disease conditions. Hematology - Science and Practice consists of a selection of essays which aim to inform both specialist and non-specialist readers about some of the latest advances in hematology, in both laboratory and clinic.

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