The Cancer Cathedral

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Today a biomedical scientist is not much different from a stonemason working on a gothic cathedral in medieval times. The stonemason often worked diligently on a single gargoyle for his entire life. As it usually took centuries to build the cathedral, most stonemasons never lived to see their work in the context of the finished building. Modern day scientists often do the same. However, science has been accelerating rapidly, especially since the advent of monoclonal antibodies and genetic engineering 30 years ago. The quest to understand cancer is a classic example of the construction of a modern scientific gothic cathedral, which has taken the combined efforts of many "scientific stonemasons". However, now because of the long lead time, and the quickening pace of research, we have finally completed the construction of this "cancer cathedral".

The foundations of the cancer cathedral were laid almost a half-century ago and are attributable to the work of a single young scientist, Peter Nowell of the University of Pennsylvania. In 1960 Nowell discovered that lymphocytes were capable of proliferating in response to the mitogenic plant lectin phytohemagglutinin (PHA), an extract from kidney beans used to agglutinate Red Blood Cells (RBCs)[1]. Prior to this discovery, lymphocytes were described in textbooks as differentiated, end stage cells incapable of self renewal. Of course, just prior to Nowell's report, Macfarlane Burnet had postulated that lymphocytes were the fundamental cells comprising the immune system, and as a central tenet of his Clonal Selection Theory, he proposed that lymphocytes had to proliferate so that the clones of antigen-reactive cells could expand, a change in the whole cell population necessary before an efficacious response to the antigen could occur[2].

In this very first report of lymphocyte *"blastic transformation"* and mitosis, Nowell had already made the crucial observation that although PHA was necessary to initiate mitosis, it did so after a considerable time lag of several days, thereby prompting him to speculate that some other critical factor was at work to actually promote all of the cellular changes necessary for lymphocyte proliferation[1].

Also in 1960, Nowell used his newly discovered technique of inducing normal lymphocytes to undergo mitosis to make the novel observation that cells from patients with chronic myelogenous leukemia (CML), contained an abnormal small chromosome, by comparison with the chromosomes found in PHA-stimulated normal lymphocytes[3]. This abnormal chromosome was named the *Philadelphia (Ph) Chromosome,* in anticipation of many such chromosomal abnormalities to be found, in the same way that abnormal hemoglobins had been named for the city of their first reporting.

As Nowell recounted in a recent review [4], *"Because this abnormality was present in essentially every dividing leukemic cell, and in every typical case of CML, it strongly suggested two major*

conclusions: first, that the leukemia in each patient had arisen from a single cell in which this somatic genetic change had occurred, and given the progeny of that cell a selective growth advantage for clonal expansion, ultimately leading to systemic dispersion and clinical CML; and, second, that this specific genetic change, visible microscopically, was critical to the pathogenesis of this type of leukemia."

Then, only a few years passed before there appeared two papers simultaneously in *Nature*, both of which revealed the presence of a *mitogenic activity* in the conditioned medium of alloantigen-stimulated lymphocytes [5, 6]. The mitogenic activity was christened ***Blastogenic Factor,*** and was subsequently found in media conditioned by PHA stimulation, as well as soluble protein antigen activation. Subsequently, over the next ten years there accumulated many reports of mitogenic activities found in the conditioned media of stimulated lymphocyte cultures. However, the molecular nature of these mitogenic activities remained obscure, owing primarily to the lack of readily applicable protein purification and characterization methods.

Also in the '60s, the National Cancer Institute launched a "Special Virus Cancer Program". This program was founded because of the growing evidence that viruses could cause cancer, especially RNA tumor viruses. Acellular filtrates were first demonstrated by Ellerman and Bang in 1908 to cause leukemia in chickens [7]. Soon thereafter, in 1911 the Rous Sarcoma Virus (RSV) was described by Peyton Rous as a cell-free filtrate that caused a rapidly fatal sarcoma in chickens [8].

However, it was not until 1951 that Ludwig Gross first described leukemogenic C-type RNA tumor viruses in mice, the first mammalian species [9]. Later, Charlotte Friend reported another new virus in mice that caused an erythroleukemia (1957) [10], which was followed by the discovery of the Moloney Leukemia virus, which caused a thymic lymphoma/leukemia (1960) [11], and then the Rauscher Leukemia virus, which caused an erythro/lymphoid leukemia (1962) [12], also in mice. Then, Feline Leukemia virus, which caused a lymphoid leukemia in cats, was reported in 1969 [13].

To explain how RNA viruses could cause cancer in a cell that was passed on to all of the cellular progeny, Howard Temin proposed the ***"DNA Provirus Hypothesis"*** in 1964, thereby going against "The Central Dogma" of the flow of information only from DNA to RNA to protein [14]. This led him to devise experiments that revealed RNA virus-directed DNA synthesis, and eventually to the discovery of the RNA-dependent DNA polymerase which came to be known as Reverse Transcriptase in 1970 [15]. Similar experiments were reported simultaneously by David Baltimore in the same issue of *Nature* [16].

All of these findings led Temin to refine his hypothesis to the ***"Protovirus Hypothesis"*** [17]. To explain how RNA viruses could cause tumors, he speculated that the capacity of the retroviruses to redirect the flow of genetic information from RNA to DNA and then back to RNA, allowed the virus to "pick up" bits of normal cellular DNA and incorporate it into the viral genome.

Thus, he proposed that normal cellular genes involved in fundamental cellular processes like proliferation and differentiation, were the cause of cancer. These genes are now called **cellular proto-oncogenes**.

Also in 1970 another new murine virus was reported to cause "lymphosarcomas" with also a marked increase in polymorhonuclear (PMN) leukocytes in the blood [18]. This virus, termed Abelson lymphosarcoma virus, was destined to play a major role in the cancer virus story.

In 1973 new techniques to stain chromosomes were developed that allowed a closer look at the *Ph* chromosome in cells from CML patients, which revealed that this abnormality was not only a loss of part of chromosome 22 as had been described initially, but that the missing piece seemed translocated to chromosome 9 [19]. This translocation phenomenon also proved ultimately to be very important in the evolving structure of the cancer cathedral.

Then in 1974, Arthur Pardee published a paper in the cell cycle field that was destined to become a classic [20]. Through a series of experimental approaches, he showed that fibroblasts induced to proliferate in vitro by serum underwent a serum-dependent phase in the early G_1 phase of the cell cycle, but subsequently became independent of serum at a point in late G_1, before DNA replication. The point between the serum-dependent to serum-independent transition he termed the "Restriction Point", which soon became abbreviated to the "R-point".

At about the same time, in attempt to grow leukemia cells from patients with acute myeloid leukemia (AML) so as to search for a human leukemia virus, Doris Morgan and Frank Ruscetti working in Robert Gallo's laboratory, found that conditioned media taken from PHA-stimulated normal lymphocytes supported the long-term culture of normal T lymphocytes (T cells) [21]. This discovery was reported in 1976, more than a decade after the reports of Blastogenic Factor in lymphocyte conditioned media.

The ability to culture T cells long-term in lymphocyte conditioned media made possible the creation of Friend Leukemia Virus (FLV) antigen-specific long-term Cytolytic T Lymphocyte Lines (CTLL) [22]. From these antigen-specific CTLL, the first monoclonal T cells were generated [23], which revolutionized the study of T cells.

Among other things, the ability to grow normal T cell clones indefinitely in lymphocyte conditioned media allowed the creation of a rapid (24 hours) quantitative bioassay for the T cell growth factor (TCGF) activity in the media [24], which was instrumental in a series of biochemical experiments that defined the characteristics of the TCGF molecule for the first time in 1981 [25]. Then, monoclonal antibodies (mAb) reactive with TCGF were generated, which permitted the purification of milligram quantities of a homogeneous single protein molecule with TCGF activity [26]. Also, preparation of radiolabeled TCGF led to the identification of the cell surface TCGF receptor [27], which was the first cytokine receptor to be discovered, and

as well, the identification of the first mAb reactive with the receptor [28].

All of these advances permitted for the first time a distinction to be made between Lymphocyte Activating Factor (LAF), a product of macrophages [29], and TCGF, a T cell product [30]. Experiments revealed that LAF functioned to promote mitogenesis of lymphocytes by augmenting T cell production of TCGF [31, 32]. Thus, because LAF was found to be functioning before TCGF, it was renamed Intreleukin-1 (IL1), and TCGF was renamed Interleuukin-2 (IL2), in anticipation that there might be more molecules yet to be discovered [32].

Armed with these new reagents and understanding of the function of IL2 to promote T cell cycle progression rather than the T cell antigen receptor (TCR) or LAF[30], for the first time experiments were possible to examine the critical molecular determinants responsible for T cell cycle progression at the single cell level [33]. Using the flow cytometer, it was readily demonstrated that T cell proliferation occurs as a result of a critical number of intermolecular IL2/IL2-receptor interactions at the cell surface, and that the cell appears capable of actually counting these molecular interactions, so that it only commits to the irrevocable decision of DNA replication when it has received the requisite number of receptor "hits" [34, 35]. This provided a molecular explanation of the R-point. It follows that loss of this exquisite growth factor-receptor control over the decision to divide could very well lead to autonomous growth, a.k.a. cancer[36, 37].

Also in 1976 Harold Varmus and Michael Bishop and their group presented the first evidence that the transforming gene of RSV (*v-src)* had a normal cellular gene counterpart *(c-src)*, thereby proving Temin's Proto-oncogene hypothesis [38]. Subsequently, this team of investigators as well as many other groups presented evidence for additional cellular proto-oncogenes by studies of the transforming genes of RNA tumor viruses. One in particular that is important for this story was *c-abl*, homologous to the transforming gene, *v-abl,* of Abelson Lymphosarcoma virus first defined by David Baltimore's group[39].

As more and more viral oncogenes and cellular proto-oncogenes were reported in the early 1980s, Robert Weinberg and Geoffrey Cooper reported that cellular DNA from both animal and human tumor cells could induce the formation of transformed foci in normal murine fibroblasts [40-43]. As there was no evidence of viral involvement in any of the tumor tissue, and as the DNA of various tumors induced transformation with high efficiencies, it was speculated that oncogenesis involved dominant genetic mechanisms resulting in the activation of cellular transforming proto-oncogenes [44, 45].

Also, at about this same time, both the *src* and *abl* proteins were found to have tyrosine-specific kinase activity [46, 47]. However, many proteins in the cell were found to be phosphorylated by these Protein Tyrosine Kinases (PTKs), and it was not clear which of the substrates, if any, were associated with the malignant transforming properties of these viral oncogenes and cellular proto-oncogenes.

In other efforts stemming from the NCI Special Virus Cancer Program, Bernard Poiez and Frank Ruscetti, working in Robert Gallo's laboratory using an assay for Reverse Transcriptase discovered the first human retrovirus in 1980 [48]. Designated Human T Leukemia Virus-I (HTLV-I), this was a typical retrovirus by structure, but it did not contain a viral oncogene like the acutely transforming RSV or Abelson virus. However, cells from leukemia patients infected with HTLV-I all had a marked over expression of the IL2R α-chain, thereby implicating IL2/IL2R growth stimulation in the mechanism of malignant transformation.

A significant advance in the *c-abl* story occurred in the early 1980s, when it was first reported by Gerard Grosveld's group that the human *c-abl* proto-oncogene maps within the region of chromosome 9 that is translocated to chromosome 22 to form the Ph chromosome in CML [49, 50]. Subsequently, the region of chromosome 22 that contained the rearrangements was found to be limited to 5.8 kbp, and termed the "breakpoint cluster region" (BCR) [50]. The fused RNA transcript of the BCR-ABL genes codes for a 210 kDa *bcr-abl* protein found in CML cells has constitutive PTK activity, unlike the normal *c-abl* protein (145 kDa). Thus, for the first time a viral-derived oncogene capable of inducing cancer in animals was found to play a role in genetically altered human cancer. However, there still remained a large knowledge gap between these findings and the mechanism whereby the expression of these genes caused malignant transformation.

The answer to this question had to await developments in two related fields, which involved the cell cycle and growth factor signaling. During the 1980s the cyclin proteins were discovered, first in dividing fertilized sea urchin [51] and clam [52] oocytes, then yeast [53]. Of particular importance for our discussion are the discoveries of the mammalian G_1 cyclin D proteins by several laboratories in 1991. It is noteworthy that Charles Sherr's group discovered D_1 cyclin by cloning genes induced by murine Macrophage Colony Stimulating Factor (M-CSF) [54]. Cyclin D_1 was also independently identified at the breakpoint of a human chromosomal 11 inversion in parathyroid adenomas, where its coding sequences were juxtaposed to the parathyroid hormone promoter [55]. The cyclin D_1 locus was also found to be deregulated in cancer by translocation [56], retroviral insertion [57], and gene amplification [58], implying that cyclin D_1 itself can act as a proto-oncogene [59].

Subsequently, using synchronized human T cells, the sequential IL2 induction of first cyclin D_2 and then cyclin D_3 mRNA and protein expression during mid to late G_1 was demonstrated by Julia Turner in 1993 [60]. Additional work uncovered how the cell moves through G_1 beyond the R-point into S-phase, when the G_1 Cyclin-D-dependent Kinases 4 & 6 phosphorylate the DNA-bound Retinoblastoma proteins, causing them to dissociate, thereby releasing the E2F transcription factors to coordinately promote the transcriptional activation of the genes necessary for DNA synthesis and replication (for review [61]).

Despite this insight as to the growth factor-receptor dependency of the R-

point decision, the molecular events actually triggered by the growth factor receptors that induce expression of the D-cyclins remained obscure. For example, early in the 1990s experiments showed that IL2 stimulation led to phosphorylation of many cytoplasmic proteins, some of which were phosphorylated on tyrosine residues [62]. However, the kinases responsible for these reactions remained obscure. Some growth factor receptors (e.g. EGF, PDGF) were known to contain PTKs in their cytoplasmic domains. However, the receptors for IL2 did not have PTK domains.

Even so, the experiments with IL2 and T cell growth pointed the way for similar experiments in all of the cells comprising the hematopoietic system, over the course of the 1980s. For example, it was well known clinically that bacterial infections often resulted in a rapid increase in circulating WBCs, and that hypoxia led to an increase in RBCs, but the mechanisms responsible remained unknown. The identification of the hematopoietic growth factor molecules and their receptors provided the mechanisms. In particular the work of Donald Metcalf, Nicholas Nicola and others in the identification, purification, and cloning of Granulocyte-Colony Stimulating factor (G-CSF) and its receptor are especially important for our discussion, because of their specificity for myeloid cell growth and differentiation [63-67]

After several hematopoietic growth factor molecules and their receptors had been identified, it was realized that they comprise a whole new superfamily of ligands and receptor molecules [68]. As well, none of the receptors had PTK

domains or any other enzymatic domains based upon their sequences that could be construed to initiate signaling to the cell interior. Thus, it was fortunate that Andrew Wilks and coworkers discovered a whole new family of PTKs at about this time [69, 70]. The Janus Kinases (JAK) were named after the Roman god Janus, because they contain 2 PTK domains predicted from DNA sequence. Janus was the god of gates, doors and doorways, beginnings and endings, and was usually depicted as two faces looking in opposite directions [71]. By 1994, the four JAK family of cytoplasmic/membrane associated kinases, JAK-1, 2, & 3, and Tyk2 had been discovered [72-74].

Also, at about the same time, workers interested in the mechanism of signaling gene expression by the interferons (IFN), the antiviral cytokines, uncovered new proteins associated with the IFN DNA response elements. Initially James Darnell's group isolated two new proteins, and because they were shown to play duals roles, acting both as signal transducers, as well as activators of transcription, they were given the names Signal Transducers and Activators of Transcription (STAT) 1 and 2 [75-77]. They soon discovered STAT3 and STAT4 [78, 79]. Then STAT5 was discovered by Groner and colleagues to be activated by prolactin in mammary tissue of lactating sheep[80]. Subsequently, STAT5 was found to exist as two almost identical (95%) isomers, so that they were designated as STAT5a&b [81, 82], and STAT6 was identified as induced by IL4 [83].

Almost simultaneously, the George Stark and Ian Kerr groups performed a series of genetic experiments that

essentially connected the JAKs with the STATs for the first time in IFN signaling of new gene expression [84] (for review [85]). James Ihle's group then demonstrated that JAK2 is phosphorylated following the binding of erythropoietin (EPO) [86], and IL3 to their respective receptors[87].

These experiments provided the impetus to see if the JAK/STAT pathways were involved in transferring the IL2/IL2R signal to the nucleus. Early on it had been established that IL2 stimulation, but not stimulation via the T cell antigen Receptor (TCR), promotes the expression of the cellular proto-oncogene *c-myb* [88], and additional experiments had identified several new genes activated solely by the IL2/IL2R interaction [89]. Thus, when approached directly, it was found that the TCR does not lead to JAK/STAT activation, but that IL2 activates JAK1 and JAK3, which promote the tyrosine phosphorylation of two new STAT proteins [90] that eventually were identified as STAT5a and STAT5b [91, 92].

As early as 1986 a natural product, erbstatin, was identified from an actinobacteria that inhibited the autophosphorylation of the Epidermal Growth Factor (EGF) receptor at concentrations in the low μM range [93]. Subsequently, using the structure of erbstatin as a base, Alexander Levitzki and co-workers synthesized a series of compounds that inhibited the autophosphorylation of the EGF receptor at low μM concentrations, and as well, inhibited EGF-dependent cell proliferation at equivalent concentrations [93]. In addition, this group reported several BCR-ABL kinase inhibitors, all

with IC_{50} in the low μM range [94]. They speculated that the mechanism of inhibition involved interference with the kinase interaction with its substrate, rather than interference with ATP binding to the kinase [95].

With this as a background, the report of small molecular inhibitors of PTKs by Brian Druker and colleagues in 1996 was incendiary for the whole cancer field [96]. Working at about the same time as Levitzki's group, investigators at Ciba-Geigy (now Novartis) identified a lead compound by screening a large chemical library. Subsequently, a series of compounds were synthesized that were predicted to block the ATP binding site of PTKs. One compound in particular blocked in vitro substrate phosphorylation by BCR-ABL with an $IC_{50} = 25$ nM and inhibited cellular PTK activity with an $IC_{50} = 250$ nM. This compound (STI571) is now called Imatinib (Gleevec). Druker's group showed that this compound inhibits cellular proliferation *in vitro* and tumor formation *in vivo* by BCR-ABL-expressing cells without inhibiting normal granulocyte colony formation. In the phase I clinical trials of this compound, when doses of 300 mg were reached, 53 of 54 patients achieved a complete hematological response [97].

These spectacular results were breathtaking, given the lack of effective chemotherapy in this disease as well as many other cancers. However, the mechanism whereby the BCR-ABL chimeric gene product led to malignant transformation was still unexplained. Because the new drug blocked the PTK activity but did not destroy the protein, it could now be surmised that the oncogenic activity was definitely related

to the PTK activity. Even so, whether the phosphorylation of just one critical protein or many were required for transformation was a critical question.

Clues to the answer of this question were found in the cytokine signaling arena. By 2000, it had been established that IL2R signaling activates JAK1 and JAK3, which in turn phosphorylate and activate STAT5 after docking onto the phosphorylated IL2R β chain. As well, it was also established that IL2R signaling activates the expression of cyclin D2 followed by cyclin D3, and that the expression of these critical cell cycle regulators leads to the cells surpassing the R-point. However, a molecular connection between STAT5 activation and cyclin D expression still had not yet been made.

In a series of careful experiments Brad Nelson's group studied the transcriptional regulation of the cyclin D2 gene using the original IL2-dependent cloned cytotoxic T cell line CTTL-2 [23]. They reported evidence of a direct pathway from the IL2R to the cyclin D2 promoter via a mechanism involving the binding of STAT5 to specific STAT5 DNA response elements in the cyclin D2 promoter [98]. Cyclin D2 mRNA is first detectable within 3 hours following IL2 stimulation, followed by a short delay in the appearance of cyclin D2 protein, thereby indicating that cyclin D2 expression in response to IL2-stimulated STAT5 is largely regulated at the mRNA level.

Because the TCR does not activate STAT5 [90], these data indicated that antigen-induced T cell proliferation is solely driven by IL2 via an IL2R activation of STAT5, followed by

STAT5 transcriptional activation of the cyclin D2 gene. Confirmation of this pathway was subsequently derived from experiments using G_0/G_1 synchronized IL2R+ human normal T cells. Activation via the IL2R in the presence of cycloheximide revealed an immediate-early expression of cyclin D2 mRNA within 4 hours, thereby providing further evidence that IL2 promotes cyclin D2 transcription directly, without a requirement for protein synthesis [99].

Further support for the IL2-STAT5-cyclin D2-driven proliferation was derived from experiments with T cells from STAT5 gene deleted mice. T cells from STAT5-/- mice cannot proliferate in response to anti-CD3 + IL2, but upon retroviral rescue with STAT5 proliferation is restored [100, 101]. Moreover, upon removal of IL2 from Wild-Type (WT) T cells, activated STAT5 is lost and the mRNAs for cyclin D2, Cyclin D3 as well as c-Myc, all known IL2/STAT5-induced genes, disappear.

Accordingly, 5 decades after Nowell's first description of the prolonged interval of several days after the addition of PHA to the first appearance of mitosis, the entire sequence of molecular events regulating T cell proliferation in response to antigenic and mitogenic activation is known: activation via the TCR signals the expression of the IL2 gene and the genes encoding the IL2R α and β chains, a process that takes 24 hours to become maximal [33, 102]. The IL2/IL2R interaction then activates JAK1 and JAK3, which phosphorylate themselves and the IL2R β chain, providing for the docking and activation of STAT5, which takes several more hours [90]. Ultimately, the cell only

progresses through the G_1 phase of the cell cycle beyond the R-point and enters S-phase as a consequence of STAT5-dependent expression of cyclin D_2 and then cyclin D_3 [60, 98]. Thus, G_1 progression takes at least 12 hours, followed by S-phase and G_2 phase of about the same time interval, before mitosis ensues.

Recent thermodynamic studies [103] as well as new structural data on the quaternary IL2/IL2R complex by Chris Garcia's [104] and Ian Wilson's [105] groups indicate that the once the IL2/IL2R complex forms at the cell surface, it is stable, and signaling continues until the complex is internalized and degraded. Thus, there is a continuous generation of signals activating STAT5 dimerization and translocation to the nucleus, with resultant formation of stable transcriptional activating complexes and continuous expression of STAT5-rsponsive genes, particularly those responsible for cell cycle progression (D cyclins) and cell survival (BclX). The cell is capable of counting these molecular interactions, and only passes the R-point when a critical number of STAT5-signaled cyclin D2 and D3 complexes have been formed [33, 35, 37, 106, 107].

All of these data make sense with regard to the mechanism of oncogenesis promoted by an abnormal constitutively active PTK such as is formed by the BCR-ABL translocation. The BCR-ABL constitutive PTK activity circumvents the growth factor/receptor control over the molecular decision making process that determines when and if the cell divides, so that the cell becomes growth factor independent.

Soon after the discovery of the STATs, constitutively phosphorylated/activated STAT3 was shown to be required for *src*-mediated transformation by Richard Jove as well as Jacqueline Bromberg[108-112]. Over the course of the past several years many reports accumulated supporting the notion that the BCR-ABL gene product mediates malignant transformation of myeloid stem cells by usurping the normal regulatory control of G-CSF/G-CSFR of signals activating JAK2, STAT5, and cyclin D2/3 and BclX, thereby leading to autonomous cell cycle progression and cell survival [113-116]. However, experiments with STAT5a/b double gene deleted mice failed to support this contention, in that these mice were still susceptible to *v-abl* transformation [117]. Accordingly, the role of the STAT proteins in oncogenic transformation remained unclear.

As discussed in the following sections, it is now realized that the original STAT5a/b gene deleted mice still expressed N-terminally truncated STAT5 protein, as only the most N-terminal exon was deleted, but the rest of the coding regions were still in-frame. Subsequently, the investigators who constructed the original STAT5 (-/-) mice repeated their experiments and deleted the total coding regions of both STAT5a and 5b. When inoculated with Abelson Virus, these mice do not succumb to lymphosarcomas [118].

Thus, the last gargoyle of the cancer cathedral is finally now in place. Moreover, Nowell's two ground breaking reports of 1960 have now come full circle to provide for a full molecular picture of carcinogenesis. The irony is that when Nowell first looked at the

PHA blastic transformed lymphocyte cultures, he initially thought that he had discovered the cause of leukemia (personal communication). It took almost 5 decades and many scientific stonemasons to bring his two seminal discoveries to fruition, as to exactly how PHA causes normal lymphocytes to proliferate in a regulated fashion, and how the *Ph* chromosome causes malignant transformation because of the loss of growth regulation.

1. Nowell PC: **Phytohemagglutinin: An initiator of mitosis in cultures of normal human leukocytes.** *Cancer Research* 1960, **20:**462-468.
2. Burnet FM: **A modification of Jerne's theory of antibody production using the concept of clonal selection.** *Aust J Sci* 1957, **20:**67-77.
3. Nowell P, Hungerford D: **Chromosome studies on normal and leukemic human leukocytes.** *J Nat Ca Inst* 1960, **25:**85-109.
4. Nowell P: **Progress with chronic myelogenous leukemia: A personal perspective over four decades.** *Annu Rev Med* 2002, **53:**1-13.
5. Kasakura S, Lowenstein L: **A factor stimulating DNA synthesis derived from the medium of leukocyte cultures.** *Nature* 1965, **208:**794-795.
6. Gordon J, MacLean LD: **A lymphocyte-stimulating factor produced in vitro.** *Nature* 1965, **208:**795-796.
7. Ellerman V, Bang O: *Zentralbl Bakteriol Abt I* 1908, **46:**595-609.
8. Rous P: **A sarcoma of the fowl transmisable by an agent separable from the tumor cells.** *J Exp Med* 1911, **132:**397-411.
9. Gross L: **"Spontaneous" leukemia developing in C3H mice following inoculation, in infancy, with AK-leukemic extracts, or AK-embryos.** *Proc Soc Exp Biol Med* 1951, **76:**27-32.
10. Friend C: **Cell-free transmission in adult swiss mice of a disease having the character of a leukemia.** *J Exp Med* 1957, **105:**307-318.
11. Moloney J: **Biological studies on a lymphoid-leukemia virus extracted from sarcoma 37. I. Origin and introductory investigations.** *J Nat Can Inst* 1960, **24:**933-951.
12. Rauscher F: **A virus-induced disease of mice characterized by erythrocytopoiesis and lymphoid leukemia.** *J Nat Can Inst* 1962, **29:**515-532.
13. Hardy W, Geering G, Old L, de Harven E, Brodey R, McDonough S: **Feline leukemia virus: occurence of viral antigen in the tissues of cats with lymphosarcoma and other diseases.** *Science* 1969, **166:**1019-1021.
14. Temin H: **Nature of the provirus of Rous sarcoma.** *Nat Can Inst Monograph* 1964, **17:**557-570.
15. Temin H: **RNA-dependent DNA polymrease in virions of Rous sarcoma virus.** *Nature* Mizutani, S, **226:**1211-1213.
16. Baltimore D: **RNA-dependent DNA polymerase in virions of RNA tumor viruses.** *Nature* 1970, **226:**1209-1211.
17. Temin H: **The protovirus hypothesis: Speculations on the significance of RNA-directed DNA synthesis for normal development and for carcinogenesis.** *J Nat Can Inst* 1971, **46:**III-VII.

18. Abelson H, Rabstein L: **Lymphosarcoma: virus-induced thymic independent disease in mice.** *Can Res* 1970, **30:**2213-2222.

19. Rowley J: **A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Geimsa staining.** *Nature* 1973, **243:**290-293.

20. Pardee A: **A restriction point for control of normal animal cell proliferation.** *Proc Natl Acad Sci U S A* 1974, **71:**1286-1292.

21. Morgan DA, Ruscetti FW, Gallo R: **Selective in vitro growth of T lymphocytes from normal human bone marrows.** *Science* 1976, **193:**1007-1008.

22. Gillis S, Smith KA: **Long term culture of tumour-specific cytotoxic T cells.** *Nature* 1977, **268:**154-156.

23. Baker PE, Gillis S, Smith KA: **Monoclonal cytolytic T-cell lines.** *J Exp Med* 1979, **149:**273-278.

24. Gillis S, Ferm MM, Ou W, Smith KA: **T cell growth factor: parameters of production and a quantitative microassay for activity.** *J Immunol* 1978, **120:**2027-2032.

25. Robb RJ, Smith KA: **Heterogeneity of human T-cell growth factor(s) due to variable glycosylation.** *Mol Immunol* 1981, **18:**1087-1094.

26. Smith KA, Favata MF, Oroszlan S: **Production and characterization of monoclonal antibodies to human interleukin 2: strategy and tactics.** *J Immunol* 1983, **131:**1808-1815.

27. Robb RJ, Munck A, Smith KA: **T cell growth factor receptors: Quantitation, specificity, and biological relevance.** *J Exp Med* 1981, **154:**1455-1474.

28. Leonard WJ, Depper JM, Uchiyama T, Smith KA, Waldmann TA, Greene WC: **A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor.** *Nature* 1982, **300:**267-269.

29. Gery I, Gershon RK, Waksman B: **Potentiation of the T-lymphocyte response to mitogens.** *J Exp Med* 1972, **136:**128-142.

30. Smith KA: **T-cell growth factor.** *Immunol Rev* 1980, **51:**337-357.

31. Smith KA, Gilbride KJ, Favata MF: **Lymphocyte activating factor promotes T-cell growth factor production by cloned murine lymphoma cells.** *Nature* 1980, **287:**853-855.

32. Smith KA, Lachman LB, Oppenheim JJ, Favata MF: **The functional relationship of the interleukins.** *J Exp Med* 1980, **151:**1551-1556.

33. Cantrell DA, Smith KA: **The interleukin-2 T-cell system: a new cell growth model.** *Science* 1984, **224:**1312-1316.

34. Smith KA: **Interleukin-2: inception, impact, and implications.** *Science* 1988, **240:**1169-1176.

35. Smith KA: **The interleukin 2 receptor.** *Annu Rev Cell Biol* 1989, **5:**397-425.

36. Smith KA: **Cell growth signal transduction is quantal.** *Ann New York Acad Sci* 1995, **766:**263-271.

37. Smith K: **Why do cells count?** In *Nonlinear cooperative phenomena in biological systems.* Edited by Matsson L. Singapore: World Scientific Publishing Co. Pte. Ltd.; 1997: 13-19

38.   Stehelin D, Varmus H, Bishop J, Vogt P: **DNA related to the transforming gene(s) of avain sarcoma viruses present in normal avian DNA.** *Nature* 1976, **260:**170-173.

39.   Goff S, Gilboa E, Witte O, Baltimore D: **Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: Studies with cloned viral DNA.** *Cell* 1980, **22:**777-785.

40.   Shih C, Shilo B-Z, Goldfarb MP, Dannenberg A, Weinberg RA: **Passage of Phenotypes of Chemically Transformed Cells Via Transfection of DNA and Chromatin.** *PNAS* 1979, **76:**5714-5718.

41.   Cooper G, Okenquist S, Silverman L: **Transforming activity of DNA of chemically transformed and normal cells.** *Nature* 1980, **284:**418-421.

42.   Krontiris TG, Cooper GM: **Transforming Activity of Human Tumor DNAs.** *PNAS* 1981, **78:**1181-1184.

43.   Shih C, Padhy L, Murray M, Weinberg R: **Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts.** *Nature* 1981, **290:**261-264.

44.   Cooper G: **Cellular transforming oncogenes.** *Science* 1982, **218:**801-806.

45.   Weinberg R: **Fewer and fewer oncogenes.** *Cell* 1982, **30:**3-4.

46.   Collett MS, Erikson RL: **Protein Kinase Activity Associated with the Avian Sarcoma Virus src Gene Product.** *PNAS* 1978, **75:**2021-2024.

47.   Ponticelli A, Whitlock C, Rosenberg N, Witte O: **In vivo tyrosine phosphorylations of the Abelson virus transforming protein are absent in its normal cellular homologue.** *Cell* 1982, **29:**953-960.

48.   Poiesz B, Ruscetti F, Gazdar A, Bunn P, Minna J, Gallo R: **Detection and isolation of C-type retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T cell lymphoma.** *PNAS* 1980, **77:**7415-7419.

49.   Heisterkamp N, Stephenson J, Groffen J, Hansen P, de Klein A, Bartram C, Grosveld GC: **Localization of the *c-abl* oncogene adjacent to a translocation break point in chronic myelocytic leukemia.** *Nature* 1983, **306:**239-242.

50.   Groffen J, Stephenson J, Heisterkamp N, de Klein A, Bartram C, Grosveld GC: **Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22.** *Cell* 1984, **36:**93-99.

51.   Evans T, Rosenthal E, Youngbloom J, Distel D, Hunt T: **Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division.** *Cell* 1983, **33:**389-396.

52.   Rosenthal E, Tansey T, Ruderman J: **Sequence-specific adenylations accompany changes in the translation of maternal messanger RNA after fertilization of *Spinsula* oocytes.** *J Mol Biol* 1983, **166:**309-327.

53.   Solomon M, Booher R, Kirschner M, Beach D: **Cyclin in fission yeast.** *Cell* 1988, **54:**738-740.

54.   Matsushime H, Roussel M, Ashmun R, Sherr C: **Colny-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle.** *Cell* 1991, **65:**701-713.

55.   Motokura T, Bloom T, Kim H, Juppner H, Ruderman J: **A novel cyclin encoded by a *bcl1*-linked candidate oncogene.** *Nature* 1991, **350:**512-515.

56.   Withers D, Harvey R, Faust J, Melnyk O, Carey K, Meeker T: **Characterization of a candidate *bcl-1* gene.** *Mol Cell Biol* 1991, **11:**4846-4853.

57.   Lammie G, Smith R, Silver J, Brookes S, Dickson C, Peters G: **Proviral insertions near cyclin D1 in mouse lymphomas: a parallel for BCL-1 translocations in human B-cell neoplasms.** *Oncogene* 1992, **7:**2381-2387.

58.   Lammi G, fantil V, Smith R, Schuuring E, Brookees S, Michalides R, Dickson C, Arnold A, Peters G: **D11S287, a putative oncogene on chromosome q13, is amplified and expressed in squamous cell and mammery carcinomas and linked to BCL-1.** *Oncogene* 1991, **6:**439-444.

59.   Sherr C: **Mammalian G$_1$ cyclins.** *Cell* 1993, **73:**1059-1065.

60.   Turner J: **IL2-dependent induction of G1 cyclins in primary T cells is not blocked by rapamycin or cyclosporin A.** *Int Immunol* 1993, **10:**1199-1209.

61.   Nevins J, Leone G, DeGregori J, Jakoi L: **Role of the Rb/E2F pathway in cell growth control.** *J Cell Physiol* 1997, **173:**233-236.

62.   Merida I, Gaulton G: **Protein tyrosine phosphorylation associated with activation of the interleukin-2 receptor.** *J Biol Chem* 1990, **265:**5690-5694.

63.   Dexter T, Garland J, Scott D, Scolnick S, Metcalf D: **Growth factor-dependent hematopoietic precursor cell lines.** *J Exp Med* 1980, **152:**1036-1047.

64.   Nicola N, Metcalf D, Matsumoto M, Johnson G: **Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells: Identification as granulocyte colony stimulating factor.** *J Biol Chem* 1983, **258:**9017-9023.

65.   Nicola N, Metcalf D: **Binding of the differentiation-inducer, granulocyte-colony-stimulating factor, to responsive, but not unresponsive leukemic cell lines.** *PNAS* 1984, **81:**3765-3769.

66.   Souza L, Boone T, Gabrilove J, Lati P, Zsebo K, Murdock D, Chazin V, Bruszewski J, Lu H, Chen K, et al: **Recombinant human granulocyte colony-stimulating factor: Effects on normal and leukemic myeloid cells.** *Science* 1986, **232:**61-65.

67.   Fukunaga R, Ishizaka-Ikeda E, Seto Y, Nagata S: **Expression cloning of a receptor for murine granulocyte colony-stimulating factor.** *Cell* 1990, **61:**341-350.

68.   Bazan JF: **Structural design and molecular evolution of a cytokine receptor superfamily.** *PNAS* 1990, **87:**6934-6938.

69.   Wilks A: **Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction.** *PNAS* 1989, **86:**1603-1607.

70.   Wilks A, Harpur A, Kurban R, Ralph S, Zurcher G, Ziemiecki A: **Two Novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase.** *Mol Cell Biol* 1991, **11:**2057-2065.

71.   Janus: *http://enwikipediaorg/wiki/Janus_%28mythology%29*.

72.   Firmbach-Kraft I, Byers M, Shows T, Dalla-Favera R, Krolewski J: ***tyk2,*** **prototype of a novel class of non-receptor tyrosine kinase genes.** *Oncogene* 1990, **5:**1329-1336.

73. Johnston J, Kawamura M, kirken R, Chen Y-Q, Blake T, Shibuya K, Ortaldo J, McVicar D, O'Shea J: **Phosphorylation and activation of the JAK-3 Janus kinase in response to interleukin-2.** *Nature* 1994, **370:**151-153.

74. Witthuhn B, Sivennoinen O, Miura O, Lai K, Cwik C, Liu E, Ihle J: **Involvement of the JAK-3 Janus kinase in signaling by interleukins 2 and 4 in lymphoid and myeloid cells.** *Nature* 1994, **370:**153-157.

75. Fu X, Kessler DS, Veals SA, Levy DE, Darnell JE, Jr.: **ISGF3, the transcriptional activator induced by interferon {alpha}, consists of multiple interacting polypeptide chains.** *ProcNatlAcad Sci USA* 1990, **87:**8555-8559.

76. Schindler C, Fu X, Improta T, Aebersold R, Darnell JE, Jr.: **Proteins of transcription factor ISGF-3: One gene encodes the 91-and 84- kDa ISGF-3 proteins that are activated by interferon {alpha}.** *ProcNatlAcadSci USA* 1992, **89:**7836-7839.

77. Fu X, Schindler C, Improta T, Aebersold R, Darnell JE, Jr.: **The proteins of ISGF-3, the interferon {alpha}-induced transcriptional activator, define a gene family involved in signal transduction.** *PNAS* 1992, **89:**7840-7843.

78. Zhong Z, Wen Z, Darnell J: **Stat3: A STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6.** *Science* 1994, **264:**95-98.

79. Zhong Z, Wen Z, Darnell J: **Stat3 and Stat4: Members of thenfamily of signal transducers and activators of transcription.** *Proc Natl Acad Sci USA* 1994, **91:**4806-4810.

80. Wakao H, Gouilleux F, Groner B: **Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers prolactin response.** *EMBO J* 1994, **13:**2182-2191.

81. Liu X, Robinson GW, Gouilleux F, Groner B, Hennighausen L: **Cloning and Expression of Stat5 and an Additional Homologue (Stat5b) Involved in Prolactin Signal Transduction in Mouse Mammary Tissue.** *PNAS* 1995, **92:**8831-8835.

82. Mui A, Wakao H, O'Farrell A-M, Harada N, Miyajima A: **Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologues.** *EMBO J* 1995, **14:**1166-1175.

83. Hou J, Schindler U, Henzel W, Ho T, Brasseur M, McKnight S: **An interleukin-4-induced transcription factor: IL4 Stat.** *Science* 1994, **265:**1701-1706.

84. Pelligrini S, John J, Shearer M, Kerr I, Stark G: **Use of a selectable marker regulated by alpha interferon to obtain mutations in the signaling pathway.** *Mol Cell Biol* 1989, **9:**4605-4612.

85. Darnell J, Kerr I, Stark G: **Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins.** *Science* 1994, **264:** p1415-1421.

86. Witthuhn B, Quelle F, Silvennoinen O, Yi T, Tang B, Miura O, Ihle J: **JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin.** *Cell* 1993, **74:** p227-236.

87.   Silvennoinen O, Witthuhn B, Quelle F, Cleveland J, Yi T, Ihle J: **Structure of the murine Jak2 protein-tyrosine kinase and its role in interleukin 3 signal transduction.** *PNAS* 1993, **90:** p8429-8433.

88.   Stern JB, Smith KA: **Interleukin-2 induction of T-cell G1 progression and c-myb expression.** *Science* 1986, **233:**203-206.

89.   Beadling C, Johnson KW, Smith KA: **Isolation of interleukin 2-induced immediate-early genes.** *Proc Natl Acad Sci U S A* 1993, **90:**2719-2723.

90.   Beadling C, Guschin D, Witthuhn B, Ziemiecki A, Ihle J, Kerr I, Cantrell D: **Activation of JAK kinases and STAT proteins by interleukin-2 and interferon alpha, but not the T cell antigen receptor, in human T lymphocytes.** *EMBO J* 1994, **13:** p5605-5615.

91.   Hou J, Schindler U, Henzel W, Wong S, McKnight S: **Identification and purification of human Stat proteins activated in response to interleukin-2.** *Immunity* 1995, **2:**321-329.

92.   Lin J-X, Mietz J, Modi WS, John S, Leonard WJ: **Cloning of Human Stat5B.** *J Biol Chem* 1996, **271:**10738-10744.

93.   Yaish P, Gazit A, Gilon C, Levitzki A: **Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors.** *Science* 1988, **242:**933-935.

94.   Anafi M, Gazit A, Zehavi A, Ben-Neriah Y, Levitzki A: **Tyrphostin-induced inhibition of p210$^{bcr-abl}$ tyrosine kinase activity induces K562 to differentiate.** *Blood* 1993, **82:**3524-3529.

95.   Levitzki A, Gazit A: **Tyrosine kinase inhibition: An approach to drug development.** *Science* 1995, **267:**1782-1788.

96.   Druker B, Tamura S, Buchdunger E, Ohno S, Segal G, Zimmerman J, Lydon N: **Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells.** *Nat Med* 1996, **2:**561-566.

97.   Druker B, Talpaz M, Resta D, Peng B, Buchdunger E, Ford J, Lydon N, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers C: **Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia.** *N Eng J Med* 2001, **344:**1031-1037.

98.   Martino A, Holmes JH, IV, Lord JD, Moon JJ, Nelson BH: **Stat5 and Sp1 Regulate Transcription of the Cyclin D2 Gene in Response to IL-2.** *J Immunol* 2001, **166:**1723-1729.

99.   Beadling C, Smith K: **DNA array analysis of interleukin-2-regulated immediate/early genes.** *Med Immunol* 2002, **1:** 2

100. Moriggl R, Sexl V, Piekorz R, Topham D, Ihle J: **Stat5 activation is uniquely associated with cytokine signaling in peripheral T cells.** *Immunity* 1999, **11:**225-230.

101. Moriggl R, Topham DJ, Teglund S, Sexl V, McKay C, Wang D, Hoffmeyer A, van Deursen J, Sangster MY, Bunting KD, et al: **Stat5 is required for IL-2-induced cell cycle progression of peripheral T cells.** *Immunity* 1999, **10:**249-259.

102. Meuer SC, Hussey RE, Cantrell DA, Hodgen JC, Schlossman SF, Smith KA, Reinherz EL: **Triggering the T3-Ti antigen-receptor complex results in clonal T cell proliferation through an interleukin 2-dependent autocrine pathway.** *PNAS* 1984, **81:**1509-1513.

103. Rickert M, Boulanger M, Goriatcheva N, Garcia K: **Compensatory energetic mechanisms mediating the assembly of signaling complexes between interleukin 2 and its alpha, beta and gamma receptors.** *J Mol Biol* 2004, **339:**1115-1128.

104. Wang X, Rickert M, Garcia KC: **Structure of the Quaternary Complex of Interleukin-2 with Its {alpha}, {beta}, and {gamma}c Receptors.** *Science* 2005, **310:**1159-1163.

105. Stauber D, Debler E, Horton P, Smith K, Wilson I: **Crystal structure of the interleukin-2 signaling complex: paradigm for a heterotrimeric cytokine receptor.** *PNAS* 2005, **103:**2788-2793.

106. Smith K: **The quantal theory of how the immune system discriminates between "self and non-self".** *Med Immunol* 2004, **3:**3.

107. Smith K: **The quantal theory of immunity.** *Cell Res* 2006, **16:**11-19.

108. Yu C-L, Meyer D, Campbell G, Larner A, Carter-Su C, Schwartz J, Jove R: **Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the *src* oncoprotein.** *Science* 1995, **269:**81-83.

109. Bromberg JF, Horvath CM, Besser D, Lathem WW, Darnell JE, Jr.: **Stat3 Activation Is Required for Cellular Transformation by v-src.** *Mol Cell Biol* 1998, **18:**2553-2558.

110. Turkson J, Bowman T, Garcia R, Caldenhoven E, De Groot RP, Jove R: **Stat3 Activation by Src Induces Specific Gene Regulation and Is Required for Cell Transformation.** *Mol Cell Biol* 1998, **18:**2545-2552.

111. Bromberg J, Wreszcznska M, Devgan G, Zhao Y, Pestell R, Albanese C, Darnell J: ***Stat3* as an oncogene.** *Cell* 1999, **98:**295-303.

112. Bromberg J: **Stat proteins and oncogenesis.** *J Clin Invest* 2002, **109:**1139-1142.

113. Horita M, Andreu E, Benito A, Arbona C, Sanz C, Benet I, Prosper F, Fernandez-Luna J: **Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-X$_L$.** *J Exp Med* 2000, **191:**977-984.

114. Gesbert F, Griffin J: **Bcr-Abl activates transcription of the *Bcl-X* gene through STAT5.** *Blood* 2000, **96:**2269-2276.

115. Xie S, Wang Y, Liu J, Sun T, Wilson M, Smithgall T, Arlinghaus R: **Involvement of Jak2 tyrosine phosphorylation in Bcr-Abl transformation.** *Oncogene* 2001, **20:**6188-6195.

116. Wittig I, Groner B: **Signal transducer and activator of transcription 5 (STAT5), a crucial regulator of immune and cancer cells.** *Current Drug Targets: Immune, End & Metabol Dis* 2005, **5:**449-463.

117. Sexl V, Piekorz R, Moriggl R, Rohrer J, Brown MP, Bunting KD, Rothammer K, Roussel MF, Ihle JN: **Stat5a/b contribute to interleukin 7-induced B-cell precursor expansion, but abl- and bcr/abl-induced transformation are independent of Stat5.** *Blood* 2000, **96:**2277-2283.

118. Hoelbl A, Kovacic B, Kerenyi MA, Simma O, Warsch W, Cui Y, Beug H, Hennighausen L, Moriggl R, Sexl V: **Clarifying the role of Stat5 in lymphoid development and Abelson-induced transformation.** *Blood* 2006, **107:**4898-4906.