

## Donald Metcalf 1929–2014\*

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Donald Metcalf was one of Australia's most distinguished medical researchers and is acknowledged internationally as the father of the modern field of haemopoietic growth factors. He defined the hierarchy of haemopoietic progenitor cells, purified and cloned the major molecular regulators of their growth and maturation, determined their mechanisms of action and participated in their development for clinical use in cancer patients. He received numerous awards and distinctions during his career, but was most pleased by the fact that his life's work improved human health.

### Early Days

Donald Metcalf was born on 26 February 1929 in Mittagong, NSW, the middle child with older and younger sisters, Rosalind and Beryl (Fig. 1). His father, Donald Davidson Metcalf, was the son of a Scottish migrant and his mother, Enid Victoria née Thomas, came from a wealthy farming family in Shepparton, Victoria. They were both associated with the education department and consequently moved from town to town through rural NSW every two to three years, including living in Womboota, Kingsvale, Goulburn, Wallerawang, Inverell, Lithgow and Tamworth. This made it difficult for young Donald to form lasting friendships with his schoolmates, and he developed a relatively solitary and self-sufficient character.

Metcalf's father was ambitious and often took night-study courses gradually moving up the ranks within the NSW education department until he eventually became headmaster of demonstration schools responsible for training new teachers in Tamworth, then Wagga then Lane Cove. Metcalf's mother was a solemn person who also taught needlework at her husband's schools and as a result Metcalf was present at these schools from a very early age, and learned to read before he was three. He was thus enrolled into first grade at age three, two years younger than his classmates. He started high school at age nine and to avoid entering university at age 14



he had to repeat his intermediate year at Lithgow and leaving year at Tamworth, being Dux of the school in both years.

The Great Depression and the Second World War came and went during Metcalf's childhood. He was somewhat isolated from these events by living in the country and having a small farm with cows and chickens. Nevertheless he was aware of the Light Horsemen training in Goulburn, the mining and arms manufacture in Lithgow and Tiger Moth flight training in Tamworth.

Metcalf studied science subjects at high school including mathematics and chemistry but it is unclear what led him to enrol in the medical course at Sydney University in 1945 just before his seventeenth birthday. Perhaps there was some parental influence for him to join a respectable profession. This was the first intake after the war, and the numbers were swollen to 650 with returned service personnel and others looking to make a new career. With such large classes personal tuition and access to human dissection was minimal, and Metcalf found the whole time quite stressful, believing he had a poor memory for

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**Figure 1.** Donald Metcalf with his mother Enid and sisters, Rosalind and Beryl, 1935. Photograph courtesy of the family of Donald Metcalf.

the detail required in examinations. During this time he boarded at various private houses and was housemaster at Sydney Grammar School in Clovelly.

Sydney University introduced a new one-year research degree (Bachelor of Medical Science) in 1950 and Metcalf was eager to join, apparently frustrated at the lack of treatments for so many of the diseases about which he was being taught.<sup>1</sup> He joined the laboratory of Patrick de Burgh in the Department of Bacteriology to work on the ectromelia ('mouse pox') virus and the liver pathology it caused. De Burgh had served as an army medical officer in Northern Australia and New Guinea where he encountered several tropical viruses that led to his interest in the pathology of infectious diseases. It is remarkable that in the space of a few years some of Australia's most eminent medical researchers and immunologists were to pass through his laboratory including Jacques Miller, Gustav Nossal, Kay Ellem and Christopher Burrell (Fig. 2). Although the equipment and conditions in the laboratory were rather primitive, the eccentric professors (de Burgh and Hugh Ward, both of whom had studied at Harvard with the famous bacteriologist Hans Zissner), spent a lot of time discussing the latest research findings with their two students. For his opinion to be taken seriously was revelatory for Metcalf, who had felt invisible in a class of 650 medical

students. Despite not publishing any papers from his time with de Burgh, Metcalf became hooked on academic research.

Metcalf finished his medical degree with a residency at the Royal Prince Alfred Hospital (RPAH), a major teaching hospital adjacent to Sydney University. He enjoyed most working in the emergency department with the constant roll call of colourful and sometimes shady characters. It was there that he first met Josephine (Jo) Lentaigne, a young nurse who sometimes worked with him. He was taken by her 'bright eyes and her wit and liveliness'. They began seeing each other, were soon engaged and were married in 1954.

Jo's parents were John Gerald Lentaigne (a doctor) and Eileen Garvey from Bangalow, NSW, and she had three older sisters; Mary, Margaret, Ann and a younger sister Genevieve. Donald and Jo Metcalf were also to have four daughters; Katherine (Kate), Mary-Ann, Penelope and Johanna. Kate and Johanna were to take up senior positions in the Victorian legal system, Mary-Ann became a teacher and Penelope became an accomplished visual artist.

The medical residency at RPAH convinced Metcalf that he was more suited to medical research than practice, and that research was the only way to improve the appalling state of ignorance about human disease and cures. As it turned out, an executive member of the then Anti Cancer Council of Victoria, Esmond Venner (Bill) Keogh (1895–1970), was a wartime friend of Hugh Ward and approached him for suggestions regarding someone to take up the new Carden Fellowship in cancer research. Wealthy businessman and Melbourne City councillor George Frederick Carden, due to family disagreements, had bequeathed much of his money to the Council in 1947, the income from which was to be used to 'find the cause and cure of cancer'. The Council had failed to find a suitably experienced overseas researcher to take on this role and so offered the Fellowship to Metcalf in 1954. Metcalf, quite conscious of his lack of experience, offered to take on the position at half salary.

Keogh worked at CSL but was also a close friend and work colleague of the previous Director of the Walter and Eliza Hall Institute of Medical Research (WEHI), Charles Kellaway, and had formed a close working relationship with Macfarlane Burnet by the time the latter was



**Figure 2.** Metcalf, Jacques Miller and Gustav Nossal all studied in the same research laboratory in Sydney, moved to the Walter and Eliza Hall Institute in Melbourne and ‘retired’ together at an official event in 1996. Photograph courtesy of the Walter and Eliza Hall Institute.

Director of WEHI. Keogh persuaded the somewhat reluctant Burnet to house the new Carden Fellow at WEHI, and so it came to be that Metcalf moved from Sydney to Melbourne to begin his research career in earnest.

### **Early Research Career at the Walter and Eliza Hall Institute of Medical Research (WEHI)**

Metcalf’s first meeting with Burnet was not what he expected. Within the first few minutes Burnet told him that cancer was an inevitable part of the ageing process, could not be prevented, and was incurable. Anyone who worked on the disease was either a fool or a rogue! The main focus of the Institute at that time was virology and Burnet insisted that if Metcalf was to do cancer research he first had to do virology research for two years, and if that was successful then he could do cancer

research. To make matters worse, Burnet offered only primitive laboratory space in the animal house, accessible by underground tunnel through the Royal Melbourne Hospital. This arrangement added to Metcalf’s feelings of estrangement from the main research life of WEHI, and his discomfort was exacerbated by his strong allergy to mice and other experimental animals.

Metcalf obediently worked on *Vaccinia virus* using Burnet’s favoured model of growth on the choriallantoic membrane of embryonated chicken eggs, but also found time to publish papers on the age incidence of various cancers from Australian statistics,<sup>2</sup> and on pseudoneoplastic changes in the allantois following viral inoculation.<sup>3</sup> He found a way to marry virology with his true interest in blood cell cancers by studying virus-induced chicken leukaemias.

Metcalf had noted Jacob Furth’s work on chicken leukaemias, and was fascinated

by Furth's demonstration that spontaneous lymphoid leukaemia formation in AKR mice could be prevented by removing the thymus, an organ of unknown function at the time. Furth had also discovered that the incidence of several types of tumours (especially endocrine tumours), could be increased by an altered balance of target hormones. Putting this together, Metcalf began searching for factors (hormones) that might stimulate lymphomatosis in mice. First he showed that plasma from patients with chronic lymphocytic leukaemia or lymphosarcoma could induce increased lymphocyte numbers in mice (that is contained a lymphocytosis stimulating factor, LSF).<sup>4</sup> He went on to show that LSF activity was also present in thymus extracts and that serum levels of LSF were dependent on an intact thymus.<sup>4</sup> However, he did not have the resources to pursue the purification of this factor at the time and its identity still remains a mystery.

Metcalf's interest in Furth's ideas about growth factor control of cancer and the role of the thymus in leukaemia led him to undertake a sabbatical with Furth at the Children's Cancer Research Foundation, Harvard Medical School in Boston (Fig. 3). In 1956, he, Josephine and young daughter Katherine, moved to Boston where in the next two years Jo was to give birth to Mary-Ann and Penelope. Both births were at the Boston's Women's Lying-In Hospital, which in 1981 was sold to Genetics Institute, a biotechnology company that was later to develop GM-CSF. Since Furth was an experimental pathologist, Metcalf learnt his trade as a mouse pathologist with him, and also learnt about leukaemia-causing viruses in the mouse, specifically the Friend virus that causes erythroleukaemia.

During this time and upon his return to WEHI, Metcalf continued his studies on LSF and the role of the thymus in its production and in controlling lymphocyte numbers.<sup>4</sup> He showed that thymectomy in young but not old mice had profound effects on lymphocyte numbers in the periphery (spleen and lymph nodes), and that additional thymus grafts under the skin of mice acted as autonomous units regulating their own lymphocyte numbers independent of each other despite the host origin of lymphocytes in the graft—a phenomenon that Metcalf speculated arose from seeding by bone marrow-derived stem cells. One exception was during pregnancy, where the original thymus involuted but the



**Figure 3.** Metcalf with Jacob Furth at Harvard Medical School in Boston around 1956. Photograph courtesy of the family of Donald Metcalf.

grafted thymus remained unchanged. Following these observations, Metcalf made detailed examinations of the birth rates of lymphocytes in the thymus (using autoradiographic analysis of radioactive thymidine incorporation into cells), and compared this with the steady-state numbers of lymphocytes. He concluded that the majority of lymphocytes born in the thymus must also die there, a concept that few were willing to accept at the time because it seemed so wasteful. Metcalf, however, was never afraid of the truth based on his sound scientific observations and spoke it regardless of the consequences. In time it became clear that this massive cell death in the thymus reflected the very important immunological processes of positive and negative selection against self-reactivity.

While Metcalf was in Boston, Burnet transformed WEHI from a virology institute to one focussed on immunology and the host response to infection. Despite this and Metcalf's work on the thymus and lymphocytes, he remained banished to the animal house. It was not until 1966, when Gus Nossal succeeded Burnet as Director, that Metcalf was welcomed back into the fold, given new laboratories in the main building, and was even appointed as Nossal's Assistant Director. Metcalf likened this event to the Beethoven opera *Fidelio*, where the prisoners come out of the underground prison, push open the grate and emerge into the sunlight.<sup>5</sup> Only he was saved not by his wife but by a new Director!

### Discovery of Haemopoietic Colony Assays and the Colony-stimulating Factors

In the early 1950s, Ray Bradley at the Physiology Department at Melbourne University (led by Douglas 'Pansy' Wright) was studying tumour growth, and to pursue these studies spent some time in Michael Potter's group at the National Cancer Institute in Bethesda, Maryland, studying plasmacytomas. He also spent time at the Imperial Cancer Research Fund in London learning to grow transformed fibroblasts in agar. At that time it was believed that only virally transformed cells could grow as anchorage-independent clones in agar. Developments by the early 1960s in perfecting tissue culture media for mammalian cell growth (by Earle and Eagle), the use of animal sera and the demonstration by Puck that single cells could grow if the medium had been 'conditioned' by other cells all converged to set Bradley on the path of trying to grow leukaemic lymphocytes in semi-solid agar using appropriate feeder cells. To do this he used glass Petri dishes in which he applied first an underlayer of feeder cells and a top layer of leukaemic cells in agar.

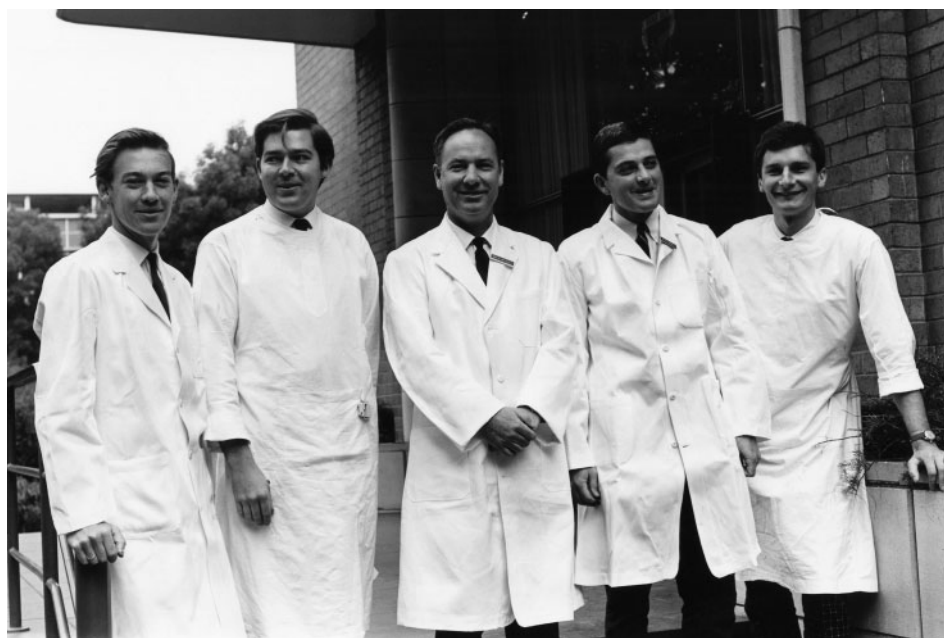
Bradley approached Metcalf to supply him with thymic lymphoma cells that develop spontaneously in AKR mice and he attempted to grow these cells using underlayers of various cell types and tissues. One of the underlayers consisted of mouse bone marrow cells and to Metcalf and Bradley's surprise colonies did develop but in the underlayer of bone marrow cells rather than the top layer of lymphoma cells. When he came across to WEHI in 1964 to show these to Metcalf, and to seek his advice on what cells were growing in the colonies, he initiated Metcalf's lifetime fascination with the power of this simple assay to define the control mechanisms of normal blood development and their transformation to leukaemias. Metcalf was staggered by the appearance of these cultures under the indirect light of a dissecting microscope—he likened the sight to one in a spaceship flying through dark space, but approaching a myriad of sparkling galaxies of varied shapes and sizes.

Bradley and Metcalf determined that these colonies consisted of monocytes (full of phagocytosed metachromatic agar granules) and polymorphonuclear granulocytes.<sup>6</sup> About the same time Dov Pluznik and Leo Sachs at the

Weizmann Institute in Rehovot, Israel described a very similar assay using mouse spleen cells in agar with feeder layers of embryonic cells, but reported that the colonies contained 'mast' cells due to the metachromatic granules in the cells.<sup>7</sup> Though these observations were independent, bitterness grew between the two groups with Sachs accusing the Australians of having 'acquired' his ideas because of his slightly earlier publication date. Unfortunately this bitterness prevented any collaboration or exchange of materials between the two groups in the ensuing years.

Following the hypothesis that excess growth factors might be associated with the development of leukaemia, Bradley and Metcalf went on to show that AKR mice with lymphoid leukaemia had elevated levels of the colony-stimulating activity (CSA) in their sera.<sup>8</sup> William (Bill) Robinson (Fig. 4), a physician from Denver doing his PhD with Metcalf, expanded these studies, as did Roger Foster when Metcalf did a sabbatical with him at the Roswell Park Memorial Institute in Buffalo, NY 1966–7. They showed that mice with other virally induced leukaemias also had elevated levels of CSA in their sera.<sup>9</sup> They also showed that the activity was filterable. These studies were important indicators to Metcalf that CSA might represent a biological regulator or growth factor that controlled blood cell growth, but in the back of his mind was always the possibility that CSA was in fact a combination of essential metabolites, secreted by feeder cells, that were missing from the culture medium or that it was a transforming virus. He therefore sought evidence of regulated levels of CSA in body fluids comparable to the altered levels of the only known blood cell regulator at the time, erythropoietin, in response to red blood cell loss or hypoxia. The elevated levels of CSA in the sera of leukaemic patients and patients with viral and other infections gave him some comfort.<sup>10</sup>

In 1967, Malcolm Moore (Fig. 4), a recent PhD graduate from Oxford University, joined Metcalf's Cancer Research Unit at WEHI to study the regulation of normal and leukaemic stem cells by the CSFs. There he became interested in the development of the haemopoietic system from the earliest embryonic stages and, with Metcalf, showed that the yolk sac was the source of haemopoietic stem cells that



**Figure 4.** Staff of the Cancer Research Unit, just after they had moved out of the institute's animal house in 1966–7. From left to right: Dr Malcolm Moore, Dr Noel Warner, Unit head Professor Donald Metcalf, Dr Bill Robinson and Dr Richard Stanley. Photograph courtesy of the Walter and Eliza Hall Institute.

would later re-populate the foetal liver and bone marrow.<sup>11</sup>

### Purification and Cloning of the CSFs

Richard Stanley (Fig. 4) came from Perth, Western Australia, in 1967 to do a PhD with Metcalf (on sabbatical at the Roswell Park Memorial Institute at Buffalo, NY, at the time of Stanley's arrival), and following the lead of erythropoietin, set about trying to identify the CSF in human urine. He determined that it was macromolecular, proteinaceous and probably not a virus.<sup>12</sup> This again allayed Metcalf's fears about the nature of CSFs but the CSF in urine stimulated almost exclusively macrophage colonies so appeared different to CSFs from other sources. Stanley performed heroically in attempting to purify this CSF from a somewhat unpleasant source, but the separation technologies available at the time were not sufficient to achieve this task. It was not until 1977, after Stanley had moved to the Ontario Cancer Institute, Toronto and later Albert Einstein College of Medicine in New York that Macrophage Colony-Stimulating Factor (M-CSF or CSF-1) from L-cell conditioned

medium was purified and shown to be highly related to human urine CSF.<sup>13</sup>

In the meantime, Metcalf went on to show that there were many different cellular sources of CSFs, and that the types of colonies stimulated varied significantly with the different sources. In particular he showed with John Parker and John Sheridan that the CSF produced by activated lymphocytes or from endotoxin-primed mouse lungs was antigenically different from M-CSF, was of a smaller size and stimulated a greater proportion of granulocyte or mixed granulocyte/macrophage colonies.<sup>14</sup> Attempts to purify this GM-CSF began with John Sheridan and were continued with Jim Camakaris.

Antony Burgess (Fig. 5) joined Metcalf's group in 1974. A protein chemist, Burgess trained in Sydney Leach's laboratory at the Biochemistry Department at Melbourne University. While working with Harold Scheraga at Cornell University in Ithaca, NY, Burgess spent time at the Weizmann Institute in Rehovot, Israel to access computer time and there became fascinated with blood cell formation after hearing a talk by Sachs. By an extraordinary coincidence



**Figure 5.** Metcalf mentored hundreds of researchers at the Walter and Eliza Hall Institute. From left to right: Dr Nicos Nicola, Dr Justin McCarthy, Dr Antony Burgess, Dr Greg Johnson, Professor Metcalf and Ms Sue Russell. Photograph courtesy of the Walter and Eliza Hall Institute.

he met Metcalf in Israel and obtained a postdoctoral position in his laboratory to purify colony-stimulating factors. Burgess went on with Metcalf to purify GM-CSF to apparent homogeneity from mouse lung-conditioned medium by 1977.<sup>15</sup> In 1976, Burgess invited Nicos Nicola (Fig. 5) (who had also trained with Syd Leach) to join him and Metcalf in the biochemical characterization of the CSFs. The three continued to fractionate CSFs from various mouse and human sources to try to define how many separate CSFs there were. Working with human placental-conditioned medium, they showed that two distinct GM-CSFs (termed  $\alpha$  and  $\beta$ ) could be separated from each other, and that they induced different kinetics of colony formation and different ratios of granulocyte to macrophage colonies.<sup>16</sup> The former was ultimately called G-CSF (indicating its preference to stimulate granulocytic colonies) and the latter simply GM-CSF.

Around this time, Metcalf returned to his core idea that growth regulator balance might be able to control the growth of cancer cells.

Using a mouse myelomonocytic leukaemic cell line (WEHI-3B) that he had generated with Noel Warner (Fig. 4) in 1969 he searched for sources of growth factors that could terminally differentiate these cells and extinguish their growth. He found that the sera of mice injected with bacterial endotoxin were a potent source of such a differentiation factor. In contrast, the differentiation activity of GM-CSF was rather weak. With Burgess, he showed that the differentiation-inducing activity did not coincide with the majority of CSF in the serum but rather with a minor sub-species of CSF that favoured granulocyte colony formation.<sup>17</sup> Since mouse lung-conditioned medium from endotoxin-injected mice was an abundant source of GM-CSF, G-CSF and the WEHI-3B differentiation-inducing activity, he and Nicola embarked on a purification strategy that would separately monitor all three activities. By 1983, G-CSF had been purified to homogeneity and shown to be identical to the WEHI-3B differentiation-inducing activity and separate from GM-CSF.<sup>18</sup>



In 1980, Burgess moved to a wing of the adjacent Royal Melbourne Hospital to become inaugural Director of the Melbourne branch of The Ludwig Institute for Cancer Research. He recruited Ashley Dunn from the Cold Spring Harbor Laboratories in Long Island, NY, and together with Nick Gough, an ex-WEHI PhD student of Jerry Adams, they embarked on a program to clone the mouse GM-CSF gene. By collaborating with Lee Hood at Caltech, who was expert at gas phase micro-amino acid-sequencing, they obtained an N-terminal peptide sequence from material purified by Burgess with Lindsay Sparrow at CSIRO. From this sequence, they generated redundant coding oligonucleotides that they used to select clones from a mouse lung cDNA library. These clones in turn were used to hybridise to mRNA from mouse lung or a T-cell line and the selected mRNA was then translated into protein in frog oocytes. The protein thus produced was tested for GM-CSF biological activity using microwell cultures of haemopoietic progenitor cells purified by flow cytometry. This allowed the molecular cloning of mouse GM-CSF cDNA and thus the capacity to generate large amounts of recombinant GM-CSF for testing *in vivo*.<sup>19</sup>

Beginning during his sabbatical at the Swiss Institute for Cancer Research in Lausanne, Switzerland in 1974–5 and continuing with Greg Johnson (Fig. 5) back in Melbourne, Metcalf showed that lectin-stimulated spleen cells produced CSA with the unique capacity to generate colonies that contained erythroid cells, megakaryocytes, eosinophils as well as granulocytes and macrophages.<sup>20</sup> This activity, termed multi-CSF, was purified by Metcalf and Rob Cutler in 1985,<sup>21</sup> but by that time it had become apparent that Multi-CSF was identical to a cytokine called interleukin-3 that had been purified by Jim Ihle in 1983<sup>22</sup> in the USA and cloned by Ian Young in 1984<sup>23</sup> at the Australian National University. This completed the formal characterization of the four species of CSFs.

### ***In vivo* Testing of CSFs and Clinical Trials**

As the recombinant CSFs became available, Metcalf participated in determining their *in vivo* actions in mice and was immensely relieved that they proved to be genuine regulators of blood cell

production as well as regulating the functional activation of granulocytes and macrophages and the migration of these cells into the tissues.<sup>24</sup>

The patent on GM-CSF was jointly owned by the Ludwig Institute for Cancer Research and the Walter and Eliza Hall Institute, and was ultimately licensed to the pharmaceutical company Schering-Plough for clinical development. Several clinical trials by competing groups and companies around the world were published from 1987 onwards, including studies by George Morstyn, Richard Fox, William Sheridan and Graham Lieschke conducted at the nearby adjacent Melbourne Hospital.<sup>25</sup> They showed that immediately following an injection of GM-CSF there was a decrease in circulating granulocytes, macrophages and eosinophils followed by an increase by 5–6 h, the kinetics suggesting that these effects were due to their egress from the blood into the tissues followed by their release from the bone marrow back into the blood. This was then sustained by increased haemopoiesis in the bone marrow. These activities of GM-CSF led to clinical trials for treating the neutropenia of AIDS patients; marrow failure in myelodysplastic syndromes and aplastic anaemia; neutropenias caused by radiation accidents and neutropenias caused by cancer chemotherapeutic treatments. It was also used to stimulate bone marrow recovery following transplantation and one of the first patients to receive this experimental treatment was the famed opera tenor Jose Carreras (Fig. 6) whose bone marrow transplant, following his leukaemia treatment, failed to take until GM-CSF was administered. This was a particular source of pride for Metcalf who, along with his wife, was a devoted opera fan. Carreras was grateful to Metcalf and offered he and his family and colleagues front row seats to the Three Tenors (Carreras, Domingo and Pavarotti) concert at the Melbourne Cricket Ground in 1997 as well as singing Happy Birthday to him on the occasion of his 70th birthday at the Melbourne Arts Centre in the same year.

Metcalf and Dunn's teams were beaten to the cloning of G-CSF by independent groups in Japan and the USA. The US pharmaceutical company AMGEN decided to conduct some of its earliest clinical trials of recombinant G-CSF in Australia to benefit from the expertise of Metcalf and his collaborators. George



**Figure 6.** Spanish opera singer Señor José Carreras was an early recipient of colony stimulating factors as part of his treatment for leukaemia, and in 1991 visited Professor Metcalf at the Walter and Eliza Hall Institute. Photograph courtesy of the Walter and Eliza Hall Institute.

Morstyn and Richard Fox again oversaw these trials at the Royal Melbourne Hospital.<sup>25</sup> As for GM-CSF they observed a rapid decrease of blood granulocytes immediately following G-CSF injection, which was followed by an even more dramatic and sustained increase in blood neutrophils than seen with GM-CSF. These early studies showed that G-CSF abrogated or reduced the period of neutropenia following single agent or combination chemotherapy for cancer resulting in fewer days on antibiotics or days in hospital and also allowed dose escalation of the chemotherapy in some cases to attempt to eradicate the tumour.

During these studies Morstyn, Metcalf and Uli Duhrsen (a visiting scientist from Germany), were examining the blood of G-CSF treated patients for haemopoietic colony-forming cells and noticed a dramatic 10- to 100-fold rise in all types of progenitor cells peaking at 4–6 days after G-CSF treatment.<sup>26</sup> This critical observation

eventually led to the realization that haemopoietic stem cells were also being mobilized into the peripheral blood after G-CSF administration. Further clinical trials led by Bill Sheridan<sup>27</sup> subsequently validated and allowed the use of G-CSF-mobilized peripheral blood stem cells, collected by apheresis, to essentially replace the more invasive procedure of bone marrow transplants for haemopoietic reconstitution following myeloablative chemotherapy. This is not only an easier and safer form of obtaining stem cells from autologous or volunteer donors, but it also appears more effective than bone marrow transplantation because of more rapid reconstitution of platelets and haemopoietic cells. Other clinical trials led by Memorial Sloan-Kettering Cancer Centre/Amgen researchers and David Dale<sup>28</sup> also revealed that G-CSF could ameliorate the neutropenia associated with severe chronic neutropenias thus transforming the lives of these children.

The results of the first-in-man trials of GM-CSF and G-CSF followed by subsequent trials for specific indications were a cause of great relief and satisfaction for Metcalf. For many years he had had niggling doubts that the CSFs might prove to be *in vitro* artifacts. In the following 25 years he would see over 20 million (mostly cancer) patients benefit from the use of CSFs (GM-CSF and G-CSF). He finally felt that his fellowship from the Anti-Cancer Council had been justified.

### CSF and Other Cytokine Receptors

Shortly after the purification of the CSFs, Metcalf, Burgess and Nicola used the precious few micrograms of purified CSFs available to radioiodinate them and use them as probes to identify and characterize their cellular receptors. The CSFs were easily damaged by oxidizing conditions so methods had to be developed that were essentially stoichiometric for incorporation of radioactive iodine. Their studies showed that several CSF receptors came in two forms—low affinity with equilibrium dissociation constants ( $K_D$ ) in the nm range and high affinity in the pM range, the latter correlating with the doses required for biological activity. Responding haemopoietic cells displayed remarkably small numbers of the high affinity receptors at the cell surface, usually in the 1–300 range.<sup>29</sup>

Metcalf used microscopic autoradiography to map the distribution of CSF receptors on different haemopoietic cells and showed that the receptor distribution faithfully matched the cell types known to respond to each type of CSF.<sup>29</sup> During these studies it became apparent that microscopic autoradiography could be a very powerful technique to detect cells that had acquired a CSF receptor by cDNA library expression screening. He, Nicola and David Gearing used this technique to screen a human placental cDNA library in COS cells and were able to clone a low affinity form of the human GM-CSF receptor.<sup>30</sup> By comparing its sequence to other recently cloned cytokine receptors they were able to define the unique sequence characteristics of this family of receptors. One of these defining sequences was a tryptophan-serine repeat (WSXWS) in the extracellular domain that was particularly useful to define unique coding DNA sequences for cytokine receptors. Metcalf and Douglas

Hilton used this method to clone additional new cytokine receptors, including the interleukin 11<sup>31</sup> and interleukin 13<sup>32</sup> receptors and showed that in each case the low affinity form of the receptor was converted to high affinity by association with additional receptor subunits. They also generated neutralising antibodies against the GM-CSF<sup>33</sup> and interleukin 13<sup>34</sup> receptors that are in clinical development for treating inflammatory and allergic diseases.

### Leukaemia Inhibitory Factor

While G-CSF was clearly the most potent cytokine stimulating differentiation of WEHI-3B cells, work from Leo Sach's group in Israel using a different mouse myeloid leukaemic cell line (M1) pointed to a differentiation-inducing factor with different properties to G-CSF. Metcalf, Douglas Hilton and Nicola imported the M1 cell line from Japan and undertook head-to-head comparisons with WEHI-3B cells in response to G-CSF, other cytokines and media conditioned by various cell lines. M1 cells did not respond to G-CSF or other CSFs, but instead a factor present in Kreb's ascites cell-conditioned medium powerfully differentiated the M1 cells.<sup>35</sup> In fact at high concentrations of this putative factor M1 cells failed to form colonies at all so this novel activity was termed Leukaemia Inhibitory Factor (LIF). Having learnt from the many years purifying CSFs the group rapidly purified, sequenced (with Richard Simpson), cloned (with David Gearing and Nick Gough) and patented LIF.<sup>35</sup>

Shortly thereafter, Lindsay Williams (originally from the European Molecular Biology laboratory in Heidelberg, Germany, but at that time working with Ashley Dunn at the Ludwig institute in Melbourne), Nicholas Gough and Metcalf showed that LIF had precisely the opposite effect on embryonic stem cells—it was the long sought after factor that maintained them in a totipotent state and prevented differentiation into mature cell lineages.<sup>36</sup> This surprising pleiotropy was reinforced in the next few years when various biological activities were purified and/or cloned and shown to be identical to LIF<sup>37</sup>. These included adipocyte lipoprotein lipase activity, cholinergic neuronal differentiation factor, inducers of the acute phase response in liver hepatocytes, and a proliferation-inducing factor for myeloid DA1 cells. LIF was also shown



**Figure 7.** Celebrating 50 years as a medical researcher in 2004 at a function at Government House organized by the Cancer Council Victoria. Metcalf (left) is joined by his four daughters (Penelope, Johanna, Kate and Mary-Ann, left to right) and wife Josephine (centre). Photograph courtesy of the Walter and Eliza Hall Institute.

to have effects on osteoblasts to stimulate bone formation and remodelling, to stimulate adrenocorticotrophic hormone release from the anterior pituitary during stress responses and to have an essential role in fertility by being required for blastocyst implantation into the endometrium and for the proper development of the placenta.<sup>38</sup>

### Suppressors of Cytokine Signalling (SOCS Proteins)

The powerful differentiation and clonal suppression of M1 cells by LIF and, as subsequently shown, interleukin 6 (IL6) made this cell line an ideal screening system for inhibitors of LIF or IL6 action. Hilton, Robyn Starr and Metcalf used M1 colony growth in agar in the presence of IL6 to screen cDNA libraries for expressed proteins that would prevent clonal suppression by IL6. They found such a protein and called it suppressor of cytokine signalling 1 (SOCS1).<sup>39</sup> Overexpression of SOCS1 inhibited the actions of many cytokines that used the Janus tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway.

The group ultimately discovered eight members of the SOCS family (CIS and SOCS1–7) each of which contained a central SH2 domain (that recognizes tyrosine phosphorylated protein sequences) and a conserved C-terminal domain called the SOCS box.<sup>40</sup> In conjunction with Jian-Guo Zhang and Richard Simpson, the group showed that the SOCS box recruited elongins B and C and Cullin 5 to form an E3 ubiquitin ligase complex that ubiquitinated and targeted for proteasomal destruction any protein bound to the SH2 domain.<sup>41</sup> In addition, two of the SOCS proteins (SOCS1 and SOCS3) contained an N-terminal sequence that could directly inhibit the catalytic activity of JAKs by binding in the substrate recognition domain of JAKs.<sup>42</sup>

Despite the apparent broad specificity of SOCS proteins in inhibiting various cytokines, gene deletion studies in mice conducted by Warren Alexander and Metcalf revealed significant physiological specificity. Loss of SOCS1 caused peri-natal lethality due to massive inflammation in the liver that could be abrogated by concomitant loss of interferon  $\gamma$ .<sup>43</sup> Loss of SOCS3 was embryonic lethal due to a failure of



**Figure 8.** Metcalf, escorted by Nicos Nicola and wife, Josephine, passes through a guard of honour of appreciative staff at WEHI celebrating his career shortly before his death. Photograph courtesy of the Walter and Eliza Hall Institute.

placentation that could be prevented by breeding the SOCS3 knockout mice to have only a single copy of the LIF gene.<sup>44</sup> Selective loss of SOCS3 in haemopoietic cells showed altered responsiveness only to the cytokines G-CSF and IL6.<sup>45</sup> Loss of SOCS2 resulted in gigantism due to enhanced growth hormone signalling<sup>46</sup> while the loss of other SOCS genes led to relatively milder phenotypes.

### Later Days

After turning sixty-five, Metcalf retired from his positions as Division Head and Assistant Director at WEHI, but there was no reduction to his contributions to science (Fig. 7). He continued to arrive at work at 6:30 a.m. although he left work a little earlier than in his younger days. As for most of his career he spent his days scoring haemopoietic colony assays, re-cloning colonies and acted as resident mouse pathologist to WEHI, systematically scanning thousands of tissue sections for every genetically modified mouse strain or mouse leukaemia model created

at the Institute. This gruelling workload bent over a microscope meant he suffered from painful back problems throughout his career, but he was never more satisfied than being made to work long and hard hours, and was never happy unless there was sufficient work to keep him occupied.

In 2014, upon the return flight from an educational cruise Metcalf felt considerable discomfort and sought a medical opinion when back in Melbourne. His symptoms were eventually determined to be due to disseminated pancreatic cancer, a disease with a very poor prognosis. Metcalf soldiered on at work while undergoing treatment until he was too unwell to work at the institute (Fig. 8). Even then he requested that his favourite microscope be transported to, and installed in, his home so he could continue to provide data to his various collaborators. Slides were ferried to him each day from WEHI and the return trip consisted of precise and detailed pathological notes on the examined material.

Eventually Metcalf had to move into hospice care, where he was visited daily by his close

family and devoted research colleagues from WEHI. To the end, he remained alert and positive. He died on 15 December 2014 at the age of 85. His service was held at the Scots Presbyterian church on 22 December, and he was buried at Melbourne General Cemetery in Parkville, about one and a half kilometres from the Institute in which he had spent almost his entire working life.

Metcalf's work has provided a powerful legacy for medical science. Throughout his career he was recognized as the outstanding figurehead of modern haemopoietic research and the father of the field of molecular regulation of haemopoiesis. His many young students and postdoctoral fellows have carved out important careers throughout the world, testaments to his mentorship and vision. Metcalf's achievements have been recognized at the highest levels both nationally (election to Australian Academy of Science, 1969; the Burnet Medal, Australian Academy of Science, 1993; Companion in the Order of Australia (AC), 1993; the Prime Minister's Prize for Science, 2001) and internationally (the Wellcome Prize of the Royal Society, London, 1986; the Bristol Myers Award for Distinguished Achievement in Cancer Research, USA, 1987; the Armand Hammer Prize for Cancer Research, USA, 1988; the Robert Koch Stiftung eV Prize, Germany, 1988; the Giovanni Lorenzini Prize for Basic Medical Research, USA/Italy, 1989; the Sloan Prize of the General Motors Cancer Research Foundation, USA, 1989; The Albert Lasker Clinical Medical Research Award, USA, 1993; the Kantor Family Prize for Cancer Research Excellence, USA, 1993; the Jessie Stevenson Kovalenko Medal, US National Academy of Sciences, 1994; the Gairdner Foundation International Award, Canada, 1994; the Royal Medal, Royal Society, London, 1995; The Warren Alpert Foundation Prize, Harvard Medical School, 1996; the Chiron International Award, National Academy of Medicine, Italy, 1999; the Donnan Thomas Prize, American Society of Hematology (US), 2004; Inaugural Salk Institute Medal for Research Excellence (US), 2005; and the Grand Hamdan International Award (UAE), Dubai, 2008).

Most important to Metcalf would be the fact that his research led directly to improved outcomes for millions of cancer patients worldwide. He always believed, and often 'preached', that

the use of discoveries to help patients was the only purpose of medical research.

## Bibliography

A full bibliography of scientific publications by Donald Metcalf is available online as Supplementary Material to this paper.

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