Joseph R. Bertino: In Memoriam (1930–2021)

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Few individuals define the physician scientist as well as Joseph R. Bertino, MD, FAACR, who died October 11 surrounded by family and friends. He was 91.

His commitment to patients can best be summed up by his own words, "When I went to medical school, I took an elective in hematology. At the same time, my brother's child came down with acute leukemia. This beautiful kid, 3 years old, died of leukemia. That made me think about a career in cancer research, hematologic malignancies, and it was a stimulus for me to continue in that area."

Joe's legacy and career journey are inextricably linked to the history of cancer chemotherapy and the training of medical oncologists. When Milton Winternitz became Yale's fifth dean in 1920, little could he imagine what would follow. Enlisted in the U.S. Armed Forces during World War II, he established a center for the study of toxic war gases. The attack on allied ships in the Italian port of Barre on December 2, 1943, spilled mustard chemicals into the oily water covering the sailors who dove for safety; many died. An investigation by Lieutenant Colonel Stewart Francis Alexander showing that these chemicals were not only skin vesicants but also had destructive effects on the lymphoid and hematopoietic systems caught the attention of Colonel Cornelius P. "Dusty" Rhoads, who served as head of New York's Memorial Hospital for the Treatment of Cancer and Allied Diseases and believed that mustard derivatives might be used to treat cancer. In 1945, he persuaded General Motors' Alfred P. Sloan and Charles F. Kettering to fund the Sloan Kettering Institute for Cancer Research to develop medicines to treat patients with cancer.

Winternitz shared Alexander's observations with two Yale assistant professors, Alfred Gilman and Louis Goodman, who began top secret studies on the effects of nitrogen mustard, a more stable derivative of sulfur mustard, on murine lymphomas and demonstrated the first signs of activity in patients. In 1949, Mustargen (mechlorethamine) became the first chemotherapeutic drug approved by the FDA.

From this early work on chemotherapy arose the need for physicians trained in both pharmacology and medicine. The Yale School of Medicine formed a division of Oncology and Chemotherapy, the first of what is today known as Medical Oncology. Initially led by Paul Calabresi, Bertino was appointed in 1966 and served for 20 years as the chief and became the first director of the Yale Cancer Center in 1973. With his colleagues in the departments of Medicine and Pharmacology, he developed today's concept of translational research, that is, applying laboratory discoveries to clinical medicine. Joe left Yale in 1986 and served until 2002 as chairman of the Molecular Pharmacology and Therapeutics Program at the Sloan Kettering Institute for Cancer Research, the institute originally conceived of by Dusty Rhoads.

I, a former Bertino lab fellow at Yale, recruited Joe along with several of his former Yale trainees and associates in 2002 to help build the Rutgers Cancer Institute of New Jersey, the state's first and only NCI designated comprehensive cancer center, where he served as University Professor of Medicine and Pharmacology and Chief Scientific Officer until his death.

Bertino's major contributions are best appreciated in the context of today's vernacular, including cancer metabolism, gene editing, molecular mechanisms of drug sensitivity and resistance, clinical and translational research, and drug discovery.

Cancer Metabolism

The Bertino lab developed and implemented the tools that elucidated the critical roles that folates play in cancer metabolism, leading to major advances in the pharmacology of antifolates in humans. These insights into folate metabolism in cancer versus normal tissues produced his pioneering clinical use of leucovorin rescue following high-dose methotrexate for patients with osteosarcoma and acute lymphocytic leukemia. His lab identified NAD kinase, encoded by *NADK*, as a target for anticancer drug development that protects tumor cells from reactive oxygens species and mediates macromolecular biosynthesis. Together with the Scotto lab, Bertino discovered an activating mutation in *NADK* that enhanced tumor cell proliferation. The finding that NAD kinase mRNA is amplified in some tumor cells prompted efforts to discover inhibitors.

Molecular Mechanisms of Drug Resistance and Sensitivity

Bertino helped define the mechanisms of action and resistance to methotrexate, historically one of the most important cancer chemotherapeutic agents. While on sabbatical with Robert Schimke at Stanford, Bertino, Rod Kellems, and Fred Alt reported that increased dihydrofolate reductase (DHFR) activity in methotrexate-resistant cells was due to amplification of the *DHFR* gene, providing the first example of gene amplification as a mechanism of drug resistance. This benchmark discovery led to the appreciation that gene amplification is an important mechanism of resistance to tight binding inhibitors. He translated these insights to studies of drug resistance in patients and reported that *DHFR* amplification and impaired methotrexate uptake were major mechanisms of resistance in blast cells from patients with acute leukemia. He later described a novel mechanism of resistance to methotrexate due to mutations in the 3′ untranslated region of *DHFR* that abrogated binding of miRNA 34a, leading to increased expression of DHFR mRNA and protein.

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With colleagues at the Rutgers Cancer Institute of New Jersey, he identified new biomarkers for methotrexate and pemetrexed sensitivity. Highly proliferative breast cancer and high-grade lymphoma cells have increased mitochondrial folate enzymes and are sensitive to treatment with methotrexate. He identified mitochondrial methylenetetrahydrofolate dehydrogenase 2 as an exciting drug target as it is expressed in embryos and not in adult tissues but is overexpressed in rapidly proliferating tumors.

Gene Editing

Using early genomic tools, his laboratory created *DHFR* and thymidylate synthase (*TYMS*) genetic variants and demonstrated that these edited genes could render hematopoietic stem cells resistant to the toxic effects of methotrexate, 5-fluorouracil, and pemetrexed and protect mice from long-term toxicity.

Clinical and Translational Research

Unravelling much of the pharmacology of methotrexate and his important work on cytosine arabinoside (AraC) allowed Bertino to design and implement one of the first curative regimens for diffuse large cell lymphoma ("COMLA," cyclophosphamide, vincristine, methotrexate with leucovorin rescue and AraC). With Seth Rudnick and Robert Capizzi, he pioneered the use of high-dose AraC in the treatment of acute leukemia. His work on the pharmacologic basis for the timing of combining methotrexate and fluorouracil led to a collaboration with Bernard Fisher and the National Surgical Adjuvant Breast and Bowel Project (NSABP) to investigate "sequential methotrexate→ fluorouracil" as one of the first alkylating agent–free adjuvant chemotherapy regimens for premenopausal women with breast cancer. Bertino's work on 5-fluorouracil included the seminal discovery that this drug produced cytotoxicity by different mechanisms of action depending on the schedule of drug administration. These findings were translated into current clinical regimens to treat colorectal cancer that use both pulse and infusional fluorouracil.

Drug Discovery

By defining the mechanisms by which folates rescue cancer cells from antimetabolites and the mechanism by which antifolates are transported across cell membranes, Bertino drove the development of trimetrexate, a lipid-soluble folate analog that does not utilize the reduced folate carrier and is not cross-resistant with methotrexate. This work was translated to the clinic by his former postdoctoral fellow, Bruce Chabner, who demonstrated the activity of trimetrexate with leucovorin rescue for the treatment of toxoplasmosis. Bertino's lab also isolated and characterized carboxypeptidase G1, an enzyme that metabolizes methotrexate, that was approved by the FDA for treating patients at risk of kidney damage following high-dose methotrexate.

Awards and Honors

Joe received numerous prestigious awards and honors. He served both as President of the American Association for Cancer Research (AACR) and President of the American Association of Clinical Oncology (ASCO) and was the inaugural editor of ASCO's *Journal of Clinical Oncology*, highlighting the esteem in which he was held in the basic, translational, and clinical research communities. A few of his many awards include, American Cancer Society Research Professorship; AACR Richard and Hinda Rosenthal Award; Memorial Sloan Kettering Cancer Center Chester C. Stock Award; ASCO Karnofsky Award; American Cancer Society Medal of Honor; ASCO Statesman Award; AACR Joseph H. Burchenal Memorial Award for Outstanding Achievement in Clinical Research; Int'l Congress on Hematologic Malignancies Outstanding Medical Research Scientist Award; American College of Clinical Pharmacology Distinguished Investigator Award; AACR Lifetime Achievement Award; and inaugural Fellow of the AACR Academy.

Joe published more than 450 peer-reviewed articles and would be quick to credit his accomplishments to his collaborators and the over 50 postdoctoral fellows whom he trained from around the world.

"Dr. B" was a beloved physician, husband, and father. His grateful patients would shower him each year with Christmas gifts. He would leave the clinic looking like a big teddy bear of a Christmas tree. The world of medical oncology has lost a giant.

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