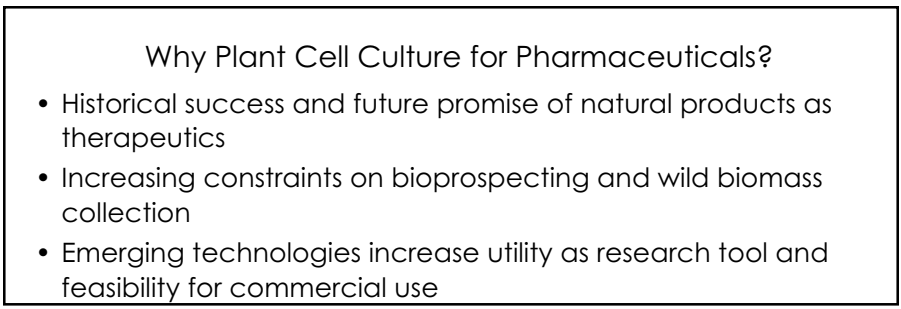
Plant Cell Culture Technology

M. DIANNE DEFURIA
Bristol-Myers Squibb Company
Princeton, N.J.

The rationale for utilizing plant cell culture for production in the pharmaceutical industry can be exemplified by the realities faced in TAXOL® development. Such a case study provides the justification for Bristol-Myers Squibb's (BMS) investment of well over $10 million to develop this technology to the scale of commercial production. BMS believes that other products will follow for plant cell culture and that other new production technologies will be developed as the need and opportunity are identified for high value products.



Why plant cell culture in the pharmaceutical industry? You are familiar with the historical successes and what we hope is the future promise of natural products with therapeutic applications. The pharmaceutical industry realizes that there are increasing constraints on bioprospecting, as well as the collection of wild biomass for production of natural product-based therapeutics. There are many emerging technologies, some of which David Evans described earlier, that have inspired people to use plant cell cultures as a research tool for drug discovery, whether from direct isolation, biotransformation, or directed fermentations. BMS is the first to look at cell culture for commercial production of a large, complex therapeutic molecule because of its high value and supply problem. The compound is paclitaxel; the active ingredient in the product, TAXOL®. This product alone motivated a large pharmaceutical company with massive

investments in all kinds of production facilities to turn to plant cell culture — a recent scientific development with little practical application.

A more in-depth look at the four reasons for using cell culture in the pharmaceutical industry reveals that the full potential of natural products in medicine has not been realized.

Success and Promise of Natural Products

- 75% of world population relies on plants for treating illness/ disease
- 25% of U.S. pharmaceutical market from plant-derived compounds, including state-of-the-art drugs, e.g. TAXOL®
- Only 2% of the >250,000 plant species have been extensively evaluated as therapeutics
- Unparalleled diversity of complex, novel molecular structures

Seventy-five percent of the world's population depends almost entirely on plants to treat illness and disease. Historically, 25 percent, and now somewhat less, of the U.S. pharmaceutical market has been based on plant-derived compounds. There are the older remedies such as digitalis, but also state-of-the-art drugs like TAXOL®. Very few of the two or three hundred species of plants have been specifically evaluated for their therapeutic benefit, and the compounds that have been derived are very novel, diverse, and complex. There are, undoubtedly, many more plant-derived compounds of medicinal value to be identified. However, one important question arises: *"Can the continued traditional screening of plant materials economically compete with new combinatorial chemistry approaches?"* The answer is not yet in, but many are betting on a combination of the two technologies.

Constraints on Bioprospecting

- Geo-political impediments to access
- Difficulty of reliable resupply
- Unrealistic expectations of many source countries
- Development vs. preservation of biodiversity, disappearance of rainforest, extinction of many species

The constraints on bioprospecting are many, (see Figure 3). BMS and many other companies have screened natural products for many years. We have all experienced the increasing geopolitical impediments to accessing new biomass for screening. This is because of the difficulties of prospecting in countries with underdeveloped scientific infrastructure, less stable governments, or other constraints. The need for resupply after an interesting compound is identified may follow years after acquisition of the original plant material. Scientific or government personnel changes may have occurred, and access for resupply is sometimes denied or impossible to accomplish. Many source countries have unrealistic expectations. Plants and their genetic material have been viewed as "green gold." Many countries believe bioprospecting within their borders offers a rare opportunity for economic development in a context of limited possibilities. This view is unrealistic based on the potential discovery of a single pharmaceutical product after 3-5 years of screening and another 10 years of high risk development by the pharmaceutical company. In addition, there is the need to balance the problem of disappearing rain forests and extinction of many plant species with the need for development in source countries. As seen in Figure 4, many technologies, from emerging genetic-engineering possibilities to evolutionary improvements in standard research techniques, have been used to increase the utility of cell culture at any scale.

Emerging Technologies

- Transgenic plants proteins (Abs, insulin)
  - — *Agrobacterium spp* vectors
  - — "Gene Gun" technology
- Improvements in analytical chemistry, robotics, and "micro research"
- Improvements in bioreactor design for enhanced mass transfer

The last underlying reason for cell culture in the pharmaceutical industry is identification of a high value product with limited options for facile commercial production. TAXOL® clearly fits into this category.

High Value Products/Supply Problem

- Skikonin—naphthoquinone for skin ailments and as a dye in cosmetic and silk industry
- Paclitaxel (TAXOL®)
  - —diterpenoid for cancer therapy

TAXOL®

Pre-CRADA TAXOL® Development

1964	Anticancer activity of Pacific yew bark extract noted
1971	Isolation and purification reported
1979	Unique mechanism of action described
1983	Clinical trials initiated
1989	Activity in refractory ovarian cancer reported

TAXOL® was identified over 30 years ago by the National Cancer Institute (NCI). They found the compound to be a drug with exciting potential for cancer therapy, but sufficient quantities for development hindered its progress for decades.

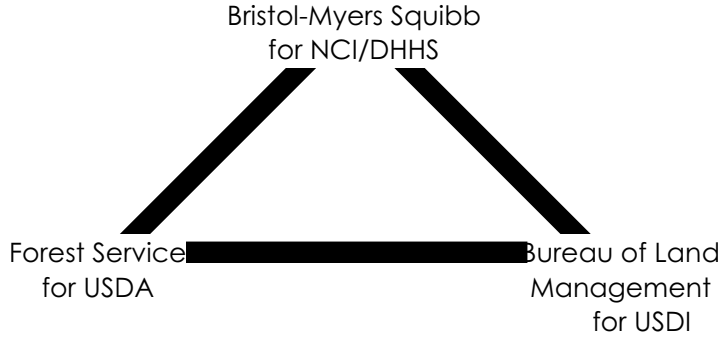
In the early 1960's, an extract of Pacific yew bark was found to contain very low concentrations of the active material. In addition, supply of the bark was extremely limited. It wasn't until 1971, prompted by this limited supply, that Dr. Monroe Wall isolated and determined the structure of TAXOL®. In 1979, Susan Horovitz identified that paclitaxel killed cancer cells in a unique way, by binding to tubulin. Paclitaxel's novel structure and unique mechanism of action made it a high priority for clinical trials by the NCI.

The progress of the clinical trials was extremely slow because only 0.5 kilogram was produced over two years. Although trials were started in 1983, it took until 1989 to demonstrate clinical activity in solid tumors. The first success was with refractory ovarian cancer. The NCI wanted to proceed, but needed a partner to further develop the drug. A competition was held to determine who in the private sector could best develop the drug. Bristol-Myers Squibb won the competition and then established cooperative agreements with multiple government agencies, not a trivial task, to continue development.

The USDA's Forest Service and the Interior Department's Bureau of Land Management assisted in an extensive bark collection program in the Pacific Northwest — mostly in large areas of Washington, Oregon, and Idaho. The government agencies developed plans to harvest the bark in a responsible way, to transfer the bark to BMS, and to minimize the impact on the environment of this huge collection to assure that the yew tree was not threatened. Bristol-Myers Squibb paid for all those activities, bought the bark, and managed the bark collection contract with Hauser Chemical Company. No one had experience with such a large collection effort, but it was well worth the effort since the drug was made available to the NCI for vastly expanded clinical trials within a few months.

TAXOL®

Cooperative Agreements



- Government Agencies—to develop annual plans, oversee bark transfer, and ecosystem research/conservation studies
- BSM—pay all government expenses, buy bark, and administer bark collection contract

The Pacific yew tree, *Taxus brevifolia*, is a small, scrubby-looking, extremely slow-growing tree. Very little was known about it — whether it existed and how many there were. The tree was considered a nuisance shrub by timber companies harvesting high-value timber like Douglas Fir. Fortunately, from spring to fall the bark of the Pacific yew was easily peeled by hand from the trunk and major limbs of the tree. Up to 1,000 people were in the woods harvesting bark from 1991- 1993. The Forest Service issued a permit for each collection bag and policed the effort closely. There was quite a bureaucracy associated with this collection and the prevention of theft. The bark was taken to collection centers where it was ground to a workable size, further milled, either dried in the sun or in low temperature tumblers, and then packed in large wooden boxes and trucked to Colorado to be chemically extracted. This process was a low-tech but high brute-force effort. It was an inefficient process, with uncontrollable quality of the raw materials, but at the time it was the only possible production process and it worked to the benefit of U.S. public health.

An alternative source was needed as quickly as possible, so BMS immediately established the multifaceted research program outlined in the following figure.

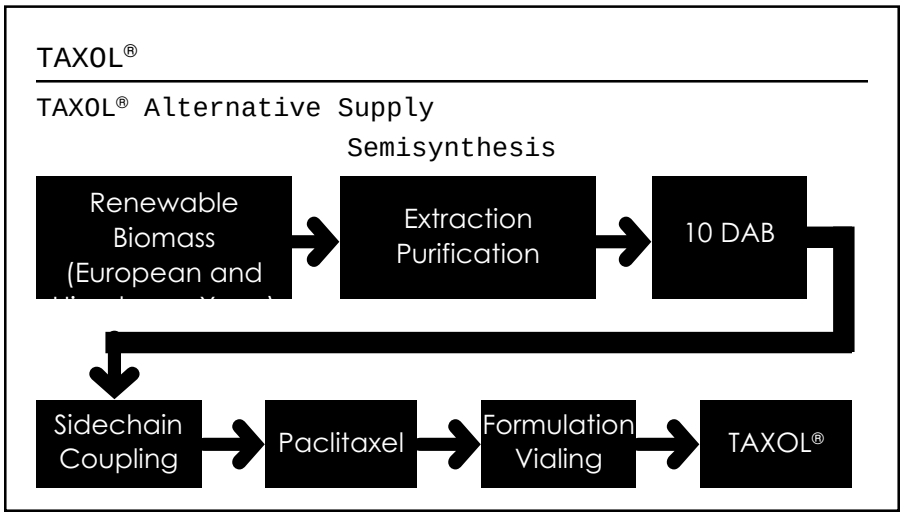
TAXOL®

Alternative Sourcing Research

- Yew plantations
  - — R&D to determine best cultivar and conditions
  - — Cultivation of biomass for commercial supply
- Renewable biomass (clippings)
  - — Precursor isolation/semi-synthesis
  - — Direct isolation of paclitaxel
- Plant cell culture
- Total synthesis ?
- Other ??

Together with the Weyerhauser Paper Products Company, several yew tree plantations were established that served two purposes — research for determination of the best cultivar for future plantations, and development of the best methods to cultivate the huge amounts of biomass that would be necessary for commercial supply. Prior to reaching these objectives, BMS had 12 million yew trees planted as a commercial source of biomass. The research effort with Weyerhauser used tens of thousands of clippings collected from ornamental nurseries all over the United States. They were hand-planted and cultivated for months in huge greenhouses built specifically for this purpose so that all growing conditions could be completely controlled. The plants were transferred to nurseries to acclimate them to the outdoors and then grown in commercial-scale nurseries. They were grown for three to five years before harvest. The initial idea was to harvest the entire plant and process it to extract either the core of the paclitaxel molecule or the intact product. But, it turned out that the important precursor 10 deacetyl baccatin III (10 DAB) was much more readily and economically available from *Taxus baccata,* which grows in Europe and Asia, than from the North American Taxus species. Therefore, commercial supplies of 10 DAB were produced in Europe for use by BMS.

Years earlier the company had licensed a process to attach the precursor 10 DAB to the active side chain, and semisynthesis proved to be a commercially viable option. The renewable biomass, originally gathered in the wild in Europe and Asia, but now cultivated in Italy, is extracted and the purified 10 DAB is shipped to Ireland where the side chain addition occurs. The purified bulk paclitaxel is then shipped to Puerto Rico for formulation and the product, TAXOL®, is shipped to our distribution centers worldwide.



While the above process is much more efficient and controllable than bark extraction, the vagaries of weather can still have a significant impact on total production and quality of the raw materials.

The plant cell culture option was brought to the attention of BMS by a small company, Phyton Catalytic. This company had been interested in developing plant cell culture specifically for the production of paclitaxel for several years. BMS agreed to sponsor their research and licensed the technology as a possible method of production.

Might the BMS collaboration with Phyton on plant culture provide an even better process? We believe the answer will be yes. Although a great deal of research has been accomplished, much development remains. Tens of thousands of yew tree explants were grown on solid nutrient media until calluses were developed. Small flask suspension cultures were used to select and determine optional growth parameters for high producing cell lines. Further research was done in the bench top scale bioreactor where it was determined quickly that paclitaxel produced in cell culture had fewer impurities than that isolated from bark and needle extracts. In addition, cell lines could be selected to produce a specific Taxane of interest.

The potential advantages of cell culture production of paclitaxel emerged quickly and can be generalized to other fermentations as well.

Advantages of Plant Cell Culture

- Environmentally benign
- Faster growth compared to plants
- Controlled, reliable supply of high quality bulk
- Quick response to variability in demand
- Culture conditions controlled easily
- Simplified downstream processing
- Novel metabolites

Plant cell culture is a more environmentally benign process and produces product faster than cultivation of biomass. It is a controlled, reliable source of high-quality material and production levels can be matched to commercial demand. The cell culture conditions are more easily controlled to assure the fermentation of the product of choice and its high quality. Downstream processing for isolation and purification of the desired product often can be simplified. This may translate into lower costs, but that has not yet been established. Cell culture provides the opportunity for additional novel metabolites to be identified that may have a broader spectrum of activity or an increased potency. These interesting possibilities have been recognized by major pharmaceutical companies other than BMS. For example, Merck and Pfizer are using cell culture for discovery of new or improved medicinal compounds, and new companies, such as Phytera, are being formed specifically for this purpose.

Today, in Arensburgh, Germany, Phyton operates the world's largest dedicated plant cell culture facility. There are cascades of fermentors from 75 to 75,000 liters, and the facility is currently being modified to comply with Good Manufacturing Process regulations required for production of human pharmaceuticals. BMS and Phyton continue to collaborate on scaling up the fermentation of new *Taxus sp.* cell lines and to develop an optimized isolation and recovery process.

Today, TAXOL® is approved for use in the therapy of both breast and ovarian cancer. New, improved treatment schedules have been developed and approved for use. The semisynthetic method for manufacturing the drug is approved and TAXOL® is currently in use in more than 50 countries worldwide. Another major market, Japan, should clear the product for marketing in 1997.

TAXOL®	Bristol-Myers Squibb

Regulatory Status

- U.S. FDA approvals
  - 12/92 ovarian cancer
  - 4/94 breast cancer
  - 6/94 improved dosage regimen
  - 10/94 semisynthetic manufacturing process
- Approved in over 50 countries worldwide

BMS expects the need to increase somewhat as the clinical research program defines new uses for the drug. The company is confident, however, in its ability to meet all patent and market demand for TAXOL®. New efficiencies will be implemented in the semisynthetic process, as well as full development and commercialization of plant cell culture within a few years.

TAXOL® is an exciting product, which in addition to offering clinical benefit to hundreds of thousands of patients, has been the sole justification for commercial development of an important technology that was little more than a laboratory curiosity for decades.