the fluorescent cDNA to the DNA microarray, where the nucleotide sequence of the cDNA matches closely the sequence of one particular DNA that is attached to the slide. Where there is matching, hybridization occurs, and the signal from hybridization is processed to take the form of an array of squares that are colored green or red. Typically, a green square means that the expression of the gene is low, while a red square means that gene expression is high. Non-binding fluorescent DNA needs to be washed away before measuring hybridized fluorescent DNA.

In published studies of clinical trials, it is typical for the study to be conducted in two parts. In part one, the researcher uses a microarray containing a huge number of genes, perhaps 5,000 genes, determines which genes show increased (or decreased) expression in patients who are helped by a drug, and determines which genes show increased (or decreased) expression in patients who are not helped by the drug. The researcher identifies which genes are most altered in the helped patients, versus in the non-helped patients. There may be about 50 genes in this group. In part two, the researcher manufactures a microarray containing these 50 genes, and then uses this microarray as a tool or device on a second group of patients. In short, tissue samples are taken from all patients before starting drug therapy, and the expression levels of the 50 genes are measured. Then, all subjects receive the same study drug. After treatment, the researcher determines if the helped patients showed a gene expression profile that was predicted (or expected) from the predetermined gene profile. If the helped patients actually did show the expected gene expression profile, the researcher publishes the results.

According to FDA's Guidance for Industry on microarrays, where a microarray is used in a regulated clinical trial, the investigator should detail the set of data that was used to discover the genes in the microarray, as well as the set of data used to validate the microarray (83). When a microarray is used, the FDA wants data on the clinical history, demographic of the human subjects used to generate both sets of data, as well as information on how the genes were chosen. Ioannidis (84) reviewed methods for validating gene arrays.

a. Microarray used in ovarian cancer – the Spentzos study

In a study of ovarian cancer, Spentzos et al. (85) acquired tumor biopsies from 68 patients and analyzed the expression of a large number of genes. Treatment involved surgery followed by chemotherapy. In general, ovarian cancer is eradicated in 70% of

[83] U.S. Dept. Health and Human Services. Food and Drug Administration. Guidance for Industry and FDA Staff. Class II Special Controls Guidance Document: gene expression profiling test system for breast cancer prognosis (May 9, 2007).

[84] Ioannidis JP. Is molecular profiling ready for use in clinical decision making? *Oncologist*. 2007;12:301–311.

[85] Spentzos D, Levine DA, Ramoni MF, et al. Gene expression signature with independent prognostic significance in epithelial ovarian cancer. *J Clin Oncol*. 2004;22:4700–4710.