

## PRESS RELEASE



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### **FOR IMMEDIATE RELEASE**

NUCLEA BIOTECHNOLOGIES, LLC WILL OFFER A NEW LABORATORY ASSAY DESIGNED TO DETECT THE GENE/PROTEIN EXPRESSION PROFILE FOR THE DETECTION OF MBCR/ABL FUSION AND C-KIT

Pittsfield, Massachusetts.....October 10, 2008.....Nuclea Biotechnologies, LLC released today that the company will be ready to offer a new laboratory assay designed to detect the gene/protein expression profile for the detection of mbcrl/abl fusion and c-kit.

### **BACKGROUND**

Chronic myeloid (or myelogenous) leukemia (CML) is characterized by new and genetically different marrow stem cells that produce increased numbers of myeloid cells (the white blood cells that normally engulf foreign matter and fight infection). The increase in CML tumor stem cells appears to suppress the normal blood cell precursors. Although the disease involves a pluripotent stem cell, it is the myeloid lineage that usually predominates.

CML is dependent on a genetic alteration that results in a fusion between the ABL1 gene on chromosome 9 and the BCR gene on chromosome 22. The genetic alteration is called the Philadelphia chromosome (Ph or Ph1) for the city where it was first described. At first this aberration was perceived as a deletion, but with the advent of better karyotypic resolution and chromosome staining methods, it was recognized as a reciprocal exchange of material between chromosomes 9 and 22, designated as t(9;22)(q34.1 ;ql 1.2) to identify the breakpoints. The corresponding molecular alteration consists of a reciprocal translocation so that most or all of the oncogene ABL1 moves from 9q34 to the BCR gene on chromosome 22 into a region known as the breakpoint cluster region (bcr). The break mABL1 is variable, although highly restricted within the gene, invariably 5' to the second exon, whereas the breakpoint in CML may vary over the entire gene. The portion of the gene where the breaks are most common is within a 5.8 kb region known as major bcr (Mbcrl).

Although the Ph chromosome is found in 90+ % of CML cases, related alterations mark some of the remaining cases of the disease. Variant or complex rearrangements that include other chromosomal sites as well as breaks at 9q34 and 22ql1.2 are observed in approximately 4 to 8% of cases. The remaining, Ph-negative cases comprise less than 5% of all CML, since more rigorous criteria for

discrimination from other myeloid dysplasias have been established. However, as many as 50% of CML patients who test negative for the Ph chromosome by conventional cytogenetics, have the Mbc<sub>r</sub>/abl rearrangement, indicating a molecular insertion of ABL1 (a masked or cryptic translocation) into the BCR gene. In these cases molecular means are required to identify the marker. Clinical and laboratory differences suggest that CML cases lacking the Mbc<sub>r</sub>/abl rearrangement at both levels may indeed have myeloproliferative disorders other than CML. Detection of the Mbc<sub>r</sub>/abl rearrangement in Ph-negative CML patients or those with variant chromosomal translocations illustrates the greater resolving power of molecular-based techniques the gene rearrangement can be detected with probe studies *in situ* or by Southern blot or PCR, but not by conventional cytogenetics.

The Ph chromosome also is observed in other diseases: in a small proportion (3-10%) of children with Acute Lymphocytic Leukemia (ALL) and in approximately 20 to 25 % of adult ALL cases where it is strongly associated with poor survival. It is even less frequent (<2%) in Acute Myeloid Leukemia (AML) and these cases may represent examples of CML where the chronic phase was undetected or very brief. Among the ALL cases where the Ph chromosome is present, the same molecular rearrangement (Mbc<sub>r</sub>) found in CML has been reported in about 50% of children and near 50% of adults. In the remaining Ph-positive cases, although the chromosomal breakpoint appears identical, at the molecular level the break in the BCR gene is proximal on chromosome 22 (nearer to the centromere) and is designated as the minor breakpoint cluster region (mbc<sub>r</sub>).

CML, ALL and AML also may be characterized by markers in addition to Ph, including c-kit (CD117). Cluster of differentiation (CD) molecules are markers on the cell surface, as recognized by specific sets of antibodies, used to identify the cell type, stage of differentiation and activity of a cell. C-kit (CD117) is the membrane receptor for stem cell factor (SCF), also known as "steel factor" or "c-kit ligand." Steel factor is a polypeptide that activates bone marrow precursors of a number of blood cells, but its receptor is also present on other cells. C-kit (CD-117) mutations in the interstitial cells of Cajal in the digestive tract are believed to be the key to gastrointestinal stromal tumors (GISTs).

## **NUCLEA ASSAY**

Nuclea has developed an *in situ* hybridization assay for the rapid and accurate identification of the major bcr/abl (Mbc<sub>r</sub>/abl) gene fusion and c-kit (CD117) amplification status in patients having or suspected of having a lymphoproliferative disorder characterized by the presence of the Mbc<sub>r</sub>/abl gene fusion and c-kit (CD117) expression. Nuclea's assay simultaneously detects both the presence of Mbc<sub>r</sub>/abl gene fusion and c-kit (CD 117) amplification in a single biological sample of patients having or suspected of having CML or another lymphoproliferative disorder using nucleic acid probes that hybridize to the BCR gene and the ABL1 gene, together with a probe or antibody specific for c-kit (CD117) expression.

Nuclea's assay is embodied in a testing kit comprising novel DNA probes complementary to the BCR gene, the ABL1 gene and the c-kit gene tagged directly or indirectly with a detectable label; detection reagents for detection of hybridized probes; and a counterstain for staining non-hybridized DNA. The testing kit also contains reagents for denaturation and/or washing. In an alternative embodiment, the testing kit may include an antibody specific for c-kit CD 117 rather than a DNA probe.

Preferably, fluorescent labels are used to detect the presence of the Ph chromosome and c-kit (CD 117) expression.

Nuclea's assay is highly specific for lymphoproliferative disorders characterized by the presence of the Ph chromosome and c-kit (CD 117), including CML, AML and ALL, and thus can be used for the differential diagnosis and/or monitoring of disease progression, as well as for predicting the efficacy of certain therapeutic agents for treating such disorders. Nuclea's assay is highly predictive of the efficacy of therapeutic agents which are inhibitors of tyrosine kinase, the BCR/ABL fusion protein and c-kit (CD117), such as imatinib mesylate (Gleevec®), dasatinib (Sprycel™) or nilotinib.

**Nuclea Biotechnologies, LLC** - Nuclea Biotechnologies, LLC is a biotechnology services company that has developed a novel technology platform to improve greatly the efficiency of diagnostics and drug discovery research. Using the Company's extensive libraries of genetic, molecular, and outcomes data and data-mining services, research professionals in pharmaceutical and life sciences companies are able to focus time and money on the most promising paths for diagnosing and treating a broad range of diseases