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## What Does a Bone Receptor have to do with Pulmonary Arterial Hypertension?

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Pulmonary arterial hypertension (PAH) is characterized by widespread obliteration of the smallest pulmonary arteries. When a sufficient number of vessels are occluded, the resistance to blood flow through the lungs increases, and the right ventricle attempts to compensate by generating higher pressure to maintain pulmonary blood flow. When the right ventricle can no longer compensate for the increased resistance, progressive heart failure occurs. All ages are affected, but the mean age at diagnosis is 36 years. Mean survival after diagnosis is 2.8 years.

PAH may be inherited and is then classified as familial PAH. Familial PAH is inherited as an autosomal dominant trait, but the average penetrance is only 20%. Each child of an affected individual has a 50% chance of inheriting the mutant allele; however, because of reduced penetrance the risk of the child actually developing PAH is only 10% (50% x 20%).

Most PAH is caused by a mutation in the gene for bone morphogenetic receptor type-2 (*BMPR2*). Bone morphogenetic protein receptor type-2 (*BMPR-2*) is a member of the transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily of cell-signaling molecules. The majority of *BMPR2* mutations leads to a truncated *BMPR-2* protein that cannot carry out its normal cell signaling function. Loss of signal transduction may result in the abnormal smooth muscle proliferation that occurs in the pulmonary arterioles of patients with PAH.

The diagnosis of familial PAH is confirmed by the presence of two or more family members with PAH or the identification of a responsible *BMPR2* mutation in a single family member. *BMPR2* mutations are detected in about 75% of individuals with familial PAH. In about 25% of families the responsible mutation has not yet been discovered. *BMPR2* mutations have been identified in 25% of individuals who represent simplex cases (i.e., a single occurrence in a family).

Of those mutations detected, 37% have been point mutations and 48% have been larger deletions or duplications in the coding region of the *BMPR2* gene. Unfortunately, no single method detects all of these mutations. DNA sequencing detects small intragenic deletions, insertions, missense, nonsense, and splice site mutations, but does not detect larger deletion/duplications. To detect the latter, multiplex ligation-dependent probe amplification is required.

To achieve maximum sensitivity both deletion/duplication analysis and DNA sequencing need to be performed. Deletion/duplication analysis has a clinical sensitivity of only 34% for familial PAH, while the combination of deletion/duplication analysis and DNA sequencing has a higher clinical sensitivity of 70% for familial PAH and 15% for idiopathic PAH. Unfortunately, the addition of DNA sequencing more than triples the cost of testing.

## Bath Salts are not just for Bathing Any More

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Bath salts are the latest designer drugs sending patients to the emergency department. Unlike traditional bath salts that are added to bath water for a relaxing soak, these drugs, which can be ingested, inhaled, or injected, contain cardiovascular and central nervous system (CNS) stimulants such as 3,4-methylenedioxypyrovalerone (MDPV) or 4-methylmethcathinone (mephedrone).

The drugs are sold over-the-counter under a variety of names such as White Dove, Red Dove, White Cloud, Cloud Nine, Ivory Wave, Ocean Snow, White Lightning, Hurricane Charlie, Charge Plus, Scarface, and Sextasy. They represent the latest in a string of designer drugs. Patients using the drugs present to the ED with signs and symptoms of acute stimulant overdose including agitation, tachycardia, and delusions or hallucinations. At least one death has been attributed to MDPV. These designer drugs are not detected by the routine urine drug screens used in most hospital laboratories.

## Laboratory Diagnosis of Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a myeloproliferative disorder comprising approximately 15-20% of adult leukemias. CML results from clonal expansion of a malignant hematopoietic stem cell carrying the Philadelphia chromosome (Ph). Ph chromosome is produced by a reciprocal translocation between chromosomes 9 and 22 that fuses the 5' exons of the *BCR* gene from chromosome 22 with *ABL* gene exons from chromosome 9.

Break-points in *ABL* occur in the first and second introns. Break-points in *BCR* occur in one of three regions: major break-point cluster region (M-bcr) involving exons b2 or b3, minor break-point region (m-bcr) involving exon e1 or micro break-point region (u-bcr) involving exon e19. Because of these different *BCR* break-points distinct fusion mRNAs of variable length including p190, p210 or p230 are generated. Most CML patients have p210 mRNA. High levels of p190 mRNA are primarily detected in acute lymphoblastic leukemia or the blast phase of CML. Low levels of p190 mRNA have been recently reported in chronic-phase CML. Historically, diagnosis and monitoring of CML required cytogenetic studies to detect the Ph chromosome. Newer and more sensitive techniques are now available for diagnosis and post therapy monitoring. A brief overview of the various techniques available for detection of these fusion mRNAs follows.

Cytogenetic testing requires culture and harvesting of cells in metaphase for diagnosis and enumeration of Ph chromosome positive cells. Disadvantages include low sensitivity (90%), longer turnaround time, and higher cost. Advantages include high specificity and detection of additional cytogenetic abnormalities, if present.

Fluorescence in situ hybridization (FISH) uses differently labeled fluorescent DNA probes that specifically bind to regions in *BCR* or *ABL*. Presence of a translocation results in fusion of these probes and change in color. First generation probes lacked specificity. Recent modifications have significantly improved the lower limit of

detection from 10% to below 0.5% abnormal cells. The major disadvantage is technical failure including loss of cells during processing. Advantages include faster turnaround time, higher sensitivity, and the ability to detect rare variants of *BCR-ABL* and masked break points that may be missed by cytogenetic testing.

	Karyotype	FISH	RT-PCR
Sensitivity for Dx	Least (90%)	Most (95%)	Less than FISH
Sensitivity for MRD	1/30 cells	1/500 cells	> 1/M cells
Quantification	≥ 10%	≥ 0.5%	≥ 1/M
Specificity	Highest	High	Lowest
Dividing cells Required	Yes	No	No
Specimen	BM	BM/PB	BM/PB
Technical failure	No dividing cells	Loss of cells	Degraded RNA
Routine TAT	72 hr	48 hr	24 hr
Unique advantage	Detects additional abnormalities	Detects masked translocation	Determines breakpoints

Qualitative reverse transcriptase-polymerase chain reaction (RT-PCR) testing uses mRNA extracted from either peripheral blood or bone marrow. Disadvantages include the inability to detect rare variants, lower specificity due to RNA cross-contamination, and false positivity. Advantages are quick turnaround time and the ability to test either bone marrow or peripheral blood. Quantitative RT-PCR is specifically designed to quantify *BCR-ABL* mRNA for monitoring disease progression. Commercially available assays use real time RT-PCR.

In summary, either karyotyping and FISH or qualitative RT-PCR can be used for diagnosis of CML. However, FISH is preferred due to its optimal sensitivity and ability to detect variants. For post-therapy monitoring, quantitative RT-PCR is the most sensitive test available.