ALL INDIA INSTITUTE OF MEDICAL SCIENCES, MANGALAGIRI

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FROM THE EDITORIAL DESK….

Dear Friends, Greetings from Department of Pharmacology and welcome to the 24th issue of ESSENCE

Clinical trials typically monitor the study participants/participants for the duration of the research study and in most cases, they are left to fend for themselves on conclusion of the trial. However, there is a case to be made to provide post-trial access to patients. This issue of ESSENCE has a very relevant article on this important aspect.

Bioinformatics is an interdisciplinary science spanning genomics, transcriptomics, proteomics, population genetics and molecular phylogenetics. The current issue also summarises the role of bioinformatics in various fields.

Use of appropriate statistical tests is the cornerstone of biomedical research and the current issue has a guest article on Tests of significance-Parametric tests.

Further, as always, the current issue has new drug approvals, interesting news from the world of medicines, crossword puzzle on “Drugs causing /Predisposing Congestive cardiac Failure and the cartoon corner.

We hope you enjoy reading it.

Jai Hind.

Editorial Team

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Post Trial Access

After the completion of the trial, the physicians or researchers will not be able to monitor the health of the study participants. Post Trial Access (PTA) refers to providing benefits to the study participants after the study has been completed. It can be in the form of continuous access to the study intervention, if it was shown to be effective, or it can be in the investments in health infrastructure. Ethical guidelines have emphasized the necessity of PTA as early as 1970s. However, the concept of PTA gained momentum with trials on HIV treatment in developing countries. A study by "AIDS Clinical Trial Group (ACTG) 076" in 1994 showed two third reduction in HIV transmission from mother to child with the use of a specific Zidovudine regimen. The regimen was costly and WHO decided to test various other cost-effective regimens to be introduced to the developing countries. Various placebo-controlled trials were conducted, funded by different sponsors that caused a discussion concerning the ethical nature of those trials. It led to concerns of PTA. Considerable discussion has taken place over the implementation of ART therapy in both HIV prevention and treatment trials.

Best example for PTA is Imatinib clinical trial. FDA granted approval to Imatinib, a very effective drug for Chronic Myeloid Leukemia (CML), in 2003. Despite the drug's proven high efficacy in the trial patients, 3600 patients were denied access to it after the study and unfortunately died. Lapatinib similarly resulted in the deaths of 28,000 women due to delays in accessing the drug. According to reports, their lifespan would have been extended by an average of eight months. Despite commitment to PTA, the CAPRISA 004 trial could not provide tenofovir gel to its participants for 2 years. Regulations caused the delay and other delayed trials include TDF 2 (12 months delay) and iPrEx (3 months delay). Therefore, it is necessary to strictly implement PTA regulations. Though there are various guidelines regarding providing PTA, most of them are very superficial and do not answer core questions as to who should or how to provide PTA.

Justifications for providing PTA:

1. Right to health: Protection of participants' rights, safety, and well-being should continue after the study is over.
2. Minimize exploitation: PTA reduces exploitation of developing-country study subjects.
3. Avoiding harm: If the study includes a chronic disease patient, continuing the intervention may benefit or discontinuing it may harm the patient.
4. Reciprocity: Participants should be compensated for their risk-taking in research.
5. Distributive justice: PTA can reduce health care disparities between resource rich sponsors and resource poor host countries.
6. Researcher participant relationship: Researchers must maintain good relationships with study participants. Before starting the study, the researcher must specify in the protocol that the participant would receive the best care afterward. The patient must be advised of post-study therapy alternatives when consenting.

Reasons against PTA

1. The high cost of conducting studies would restrict the number of new trials.
2. Funders and researchers will be discouraged by obligations, which may shift the burden from governments.
3. Countries unable to host the experiment would be unfairly disadvantaged if long-term advantages were only provided to trial participants.
4. Trial participants are in a better situation during the trial than prior to participating in study, hence they are no worse after the trial than they were before.

Various Guidelines on the PTA

Many recommendations and guidelines have been proposed in the last two decades and few of them are as follows:

1. In 2000, Declaration of Helsinki first introduced post study obligations in biomedical research.

2. WMA Helsinki(clarification) 2004: "It is necessary during study planning process to identify post-trial access to beneficial interventions or other appropriate care. Must be described in study protocol for review by ethical review committee."

3. WMA Declaration of Helsinki (revision) 2013: "In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process."

4. The UNESCO-adopted Universal Declaration on Bioethics and Human Rights (UDBHR) stated that "benefit of a trial should be provided not only to those who participate in clinical trials but also to the global community".

5. Indian guidelines on the PTA : In the ICMR revised guidelines 2006 (Ethical guidelines For Biomedical research on Human participants: ICMR 2006) under the principle of maximization of public interest and distributive justice, mentioned that: "Whereby the research or experiment and its subsequent applicative use are conducted and used to benefit all human kind and not just those who are socially better off but also the least advantaged, and in particular, the participants themselves and or the community from which they are drawn''.

Conclusion:

PTA discussions are extensive and intricate. It is still a debatable issue because there are arguments for and against granting PTA. All the concerns about PTA provision for clinical trials cannot be resolved by a single guideline or standard protocol. When deciding PTA, each clinical trial should be looked at on its own, with the type of disease, the host countries' conditions, and the expected results of the trial all being taken into account. A prior agreement reduces the feeling of exploitation of trial participants. There are still numerous obstacles that need to be surmounted. No strong argument can be made to deny PTA until we consider specific exceptional cases in which PTA plays no role. This is due to the fact that newer drugs are not always efficacious or beneficial to study participants.

References:

1. Sofaer N, Strech D. Reasons why post-trial access to trial drugs should, or need not be ensured to research participants: a systematic review. Public Health Ethics. 2011;4:160-84.

Recent new/Update from the world of medicines ….

A novel antibiotic has been discovered to target a resistant bacterium

A novel class of antibiotics that may be able to treat Acinetobacter baumannii is synthesized. In animal models, it was discovered that zosurabalpin was efficacious against pneumonia and sepsis caused by carbapenem-resistant Acinetobacter baumannii. A tethered macrocyclic peptide (MCP) that kills A. baumannii exclusively has been found by Dr. Zampaloni and colleagues. This promising candidate was named zosurabalpin which was developed by further optimizing the molecule for both efficacy and tolerability. It inhibits a key process, transport of the molecule lipopolysaccharide (LPS), by inhibiting a complex of proteins. This complex was essential for transporting LPS to the bacterial surface to create the outer-membrane structure of Gram-negative bacteria. Zosurabalpin blocks LPS transport, and the abnormal build-up of LPS in the cell kills the bacterium. The antibiotic has been evaluated in two phase I clinical trials.

Preeclampsia and preterm birth risk may be reduced by low dose of calcium

A new study led by the Harvard T.H. Chan School of Public Health and collaborators in India and Tanzania suggests that low-dose calcium (500 mg) supplementation may be just as effective as the World Health Organization's (WHO) recommended high-dose calcium supplementation (equivalent to three 500 mg pills taken throughout each day) in preventing preeclampsia and preterm birth. The study showed the incidence of preeclampsia in India was 3.0% in women taking 500 mg of calcium daily and 3.6% in women taking 1,500 mg. Preeclampsia incidence in the Tanzania experiment was 2.7% and 3.0%, respectively.

Be Cautious…. Drug Safety Alerts

S. No.	Drug	Safety Alerts
1.	Diazoxide	Necrotizing Enterocolitis
2.	Mercaptopurine	Hypoglycaemia
3.	Oral anticoagulants	Anticoagulant-related Nephropathy (ARN)
4.	Sulfamethoxazole, trimethoprim	Haemophagocytic Lymphohistiocytosis (HLH)
5.	Topiramate	Neurodevelopmental disorders in children exposed in-utero
6.	Zinc acetate	Gastric Ulcer

Bioinformatics is an interdisciplinary science spanning genomics, transcriptomics, proteomics, population genetics and molecular phylogenetics. Bioinformaticians use high throughput molecular data in comparisons between symptom-carriers (patients, animal disease models, cancer cell lines, etc.) and normal controls. The key objectives are to

1). Connect disease symptoms to genetic mutations, epigenetic modifications, and other environmental factors modulating gene expression,

2). Identify drug targets that can either restore cellular function or eliminate malfunctioning cells, e.g., cancer cells,

3). Predict or refine drug candidates that can act upon the drug target to achieve the designed therapeutic result and minimize side effects, and

4). Assess the impact on environmental health and the potential of drug resistance.

In addition to fundamental studies of the genome and molecular biology, bioinformatics has a significant influence on a wide range of biotechnology and biomedical science fields. It can be used in agricultural biotechnology, forensic DNA analysis, drug discovery and therapy, and other fields.

Genetic Diseases

Many somatic mutations linked to genetic diseases have been found by whole exome and genomic sequencing of patients with inherited disorders; these mutations could eventually serve as therapeutic targets. In bioinformatics studies on somatic mutations, the primary challenge is identifying the disease-causing mutations among the genetic variations between matched patients and normal controls. High genetic heterogeneity is seen in many disorders, like cancer, even within the cells that comprise a single tumour. Numerous somatic mutations may result from cellular failure rather than being the cause of it. Genomes are frequently searched for regulatory patterns using bioinformatic methods. These tools include the position weight matrix (PWM), which locates a known motif's genomic location, the Gibbs sampler, which finds motifs de novo, and support vector machines, which extract differences between two sets of sequences (such as motif-present and motif-absent) and use the extracted information to scan or detect motifs in genomes. The regulatory motifs may be nuclear receptor response elements, whose identification frequently results in the improvement of therapeutic targets.

Infectious Disease

Well annotated genomes are essential for target-based drug discovery against pathogens. The general bioinformatic approach involves three essential steps. The first is to identify essential genes in the pathogen as drug targets. A genome, especially a well-annotated one, can facilitate identification of such essential genes. The second step in developing drugs against pathogen is to check if such essential genes have homologues in the host. If they do, then inhibiting such essential genes in the pathogen may have adverse effect on the function of the host homologue, and we consequently need to perform sequence and structural comparisons between the pathogen and host homologues to identify unique part in the pathogen homologue to assist in the design of pathogen-specific drugs. Third, to minimize the chance of pathogen developing drug resistance, it is important for the drug to target at specific pathogen and not it’s their

non-pathogenic relatives have increasingly become the preferred source of drug targets.

Personalized Medicine

Genomic medicine, which is described as "the use of information from genomes (from humans and other organisms) and their derivatives (RNA, proteins, and metabolites) to guide medical decision-making," is frequently referred to as "personalised medicine." However, a broader definition of personalised medicine would be a preventive, participatory, personalised, and predictive healthcare paradigm that also uses technology to administer and customise care. In actuality, such a model offers a setting for implementing genomics applications (genomic medicine). Genomic medicine offers fast and precise diagnosis, genotype-based risk stratification, and personalised therapy options that have the potential to completely transform the healthcare of a person with cancer or a rare condition.

Drug Discovery

Network analysis and systems biology which include those involving gene regulation and protein-protein interactions, are built and analysed using bioinformatics. These networks contribute to our understanding of disease mechanisms and can direct the search for novel therapeutic targets. Not only can bioinformatic analysis expedite the identification of therapeutic targets and the screening and refinement of drug candidates, but it can also help characterize adverse effects and forecast drug resistance. Mechanism-based drug discovery and drug repurposing have benefited greatly from the accumulation of high-throughput data, including genomic, epigenetic, genome architecture, cistromic, transcriptomic, proteomic, and ribosome profiling data. More accurate protein-ligand docking experiments and more insightful virtual screening were made possible by the accumulation of protein and RNA structures, the development of homology modelling and protein structure simulation, and the availability of large structure databases of small molecules and metabolites.

Forensic Medicine

Forensic science uses bioinformatics to efficiently and precisely analyse DNA evidence. This raises the testing's dependability and aids in identifying persistent issues. Applications of bioinformatics can be integrated into forensic science to thoroughly review the evidence related to the crime that was committed. The study of person identification and relatedness is a component of forensic science. Databases and molecular data are being created in order to store the DNA profiles of criminals. Forensic DNA analysis focuses on two main areas: personal identity and relatedness to other individuals.

To conclude, bioinformatics tools rely on mathematical models, and no single method is suitable for every situation, their efficacy is restricted. Handling genetically identifiable information presents ethical questions that need for careful consideration of data security and privacy. The accuracy of the data may be impacted by mistakes, discrepancies, or out-of-date information found in biological databases. Because there are differences in data formats, standards, and nomenclature, integrating data from several biological databases can be difficult. However, bioinformatics, with its applications in personalised medicine and drug development, has the potential to completely transform daily living. It can be effectively used in the domains of preventive and precision medicine, which will aid in the creation of strategies for the avoidance, management, and treatment of infectious diseases.

References

Bayat A. Science, medicine, and the future: Bioinformatics. BMJ. 2002 Apr 27;324(7344):1018-22.

New Drug Approvals…

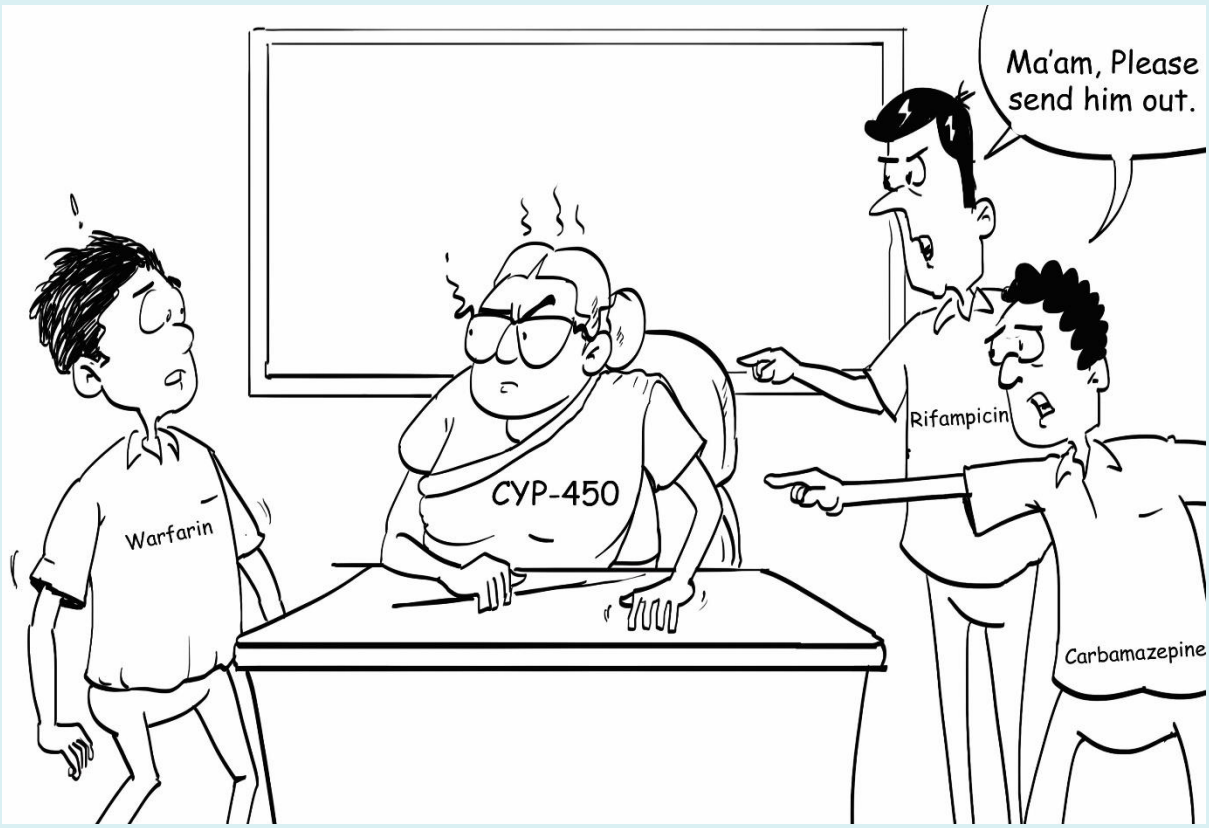
Iptacopan is a complement factor B inhibitor approved for the treatment of paroxysmal nocturnal haemoglobinuria. The recommended dose is 200 mg orally twice daily.

Capivasertib is an AKT inhibitor approved in combination with fulvestrant for the treatment of advanced hormone receptor-positive breast cancer. The recommended dose is 400 mg orally twice daily for 4 days followed by 3 days off.

Eplontersen is a transthyretin-directed antisense oligonucleotide approved for the treatment polyneuropathy of hereditary transthyretin-mediated amyloidosis. The recommended dose is 45 mg by subcutaneous injection once monthly.

Fruquintinib is a kinase inhibitor approved for the treatment of adult patients with metastatic colorectal cancer (mCRC). The recommended dose is 5 mg orally once daily for the first 21 days of 28 days cycle.

Cartoon Corner…



Excerpt from "Drug Autobiographies in Pharmacology" by Dr. Sushil Sharma

You have done a research study among a sample of 50 female and 50 male critically ill patients to study the hospital length of stay (LOS) after specific treatment. The mean and median are 60 days and 31.5 days for female and 30.9 days and 30 days for male patients. The histograms of the LOS for both genders are shown in Figures 1 & 2. You ran the two-sample 't' test (which you had heard and familiar with !!!), got a p-value of 0.04 (Hurray !!!) and you were excited as it was statistically significant. But will it stand the test of peer review?? Let's discuss.

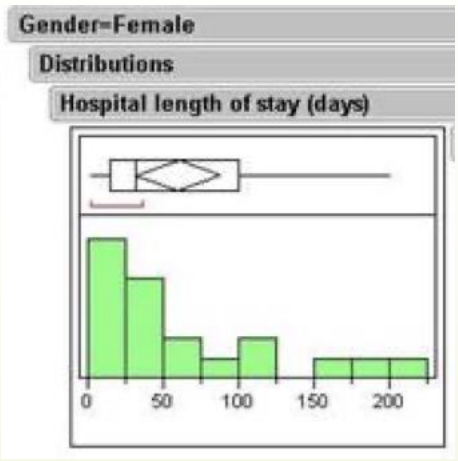


Figure 1. Female study data distribution

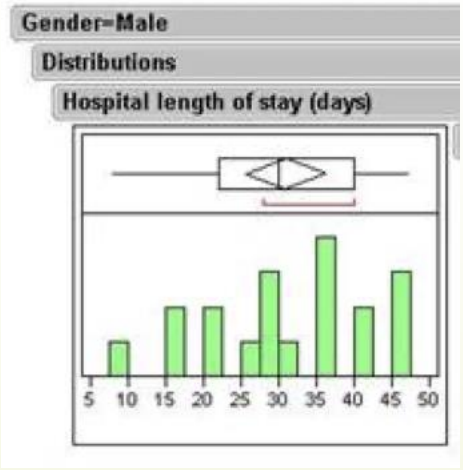


Figure 2. Male study data distribution

Biomedical research mostly involves testing the hypothesis (like the example shown above). If you have ever discussed with a Biostatistician or Community Medicine specialist regarding data analysis/hypothesis testing plan, you would have come across the terms 'parametric' and 'non-parametric' statistical tests. As you are aware, statistical tests are **statistical methods that help us reject or accept our null hypothesis**. They're based on probability distributions and can be one-tailed or two-tailed, depending on the hypotheses that we've chosen. There are other ways in which statistical tests can differ and one of them is based on their assumptions of the probability distribution that the data in question follows.

- **Parametric tests** are those statistical tests that **assume the data approximately follows a normal distribution**, amongst other assumptions (examples include z-test, t-test, ANOVA). *Important note — the assumption is that the data of the whole population follows a normal distribution, not the sample data that you're working with.*
- **Nonparametric tests** are those statistical tests that **don't assume anything about the distribution followed by the data**, and hence are also known as distribution free tests (examples include Chi-square, Mann-Whitney U). Nonparametric tests are based on the ranks held by different data points.

Parametric tests

Parametric tests are those that assume that the sample data comes from a population that follows a probability distribution — the normal distribution — with a fixed set of parameters.

Common parametric tests are focused on analyzing and comparing the mean or variance of data. The mean is the most commonly used measure of central tendency to describe data, however it is also heavily impacted by outliers. Thus, it is important to analyze your data and determine whether the mean is the best way to represent it. If yes, then parametric tests are the way to go! If not, and the median better represents your data, then nonparametric tests might be the better option.

As mentioned above, parametric tests have a couple of assumptions that need to be met by the data:

1. **Normality** — the sample data come from a population that approximately follows a normal distribution
2. **Homogeneity of variance** — the sample data come from a population with the same variance
3. **Independence** — the sample data consists of independent observations and are sampled randomly
4. **Outliers** — the sample data don't contain any extreme outliers

Parametric tests are useful when the study has continuous endpoint. The parametric test types are –

• **Z-Test**	**When you need to compare the sample's mean with a hypothesized value (which often refers to the population mean), then one sample z-test is used. The test has major requirements, such as the sample size should be more than 30, and the population's standard deviation should be known**
• **One Sample t-Test**	If either of the requirements mentioned above cannot be met, then you can use another type of parametric test known as the one-sample t-test. Here if the sample size is at least more than 15 and the standard deviation of the sample is known, then you can use this test. Here the sample distribution should be approximately normal
• **Paired (dependent) t-Test**	Paired t-test is used when from the same subject data is collected; typically before and after an event—for example, the weight of a group of 10 sportsmen before and after a diet program. Here to compare the mean of the before and after group, you can use the paired t-test. The assumptions here include groups being independent, the values of before and after belonging to the same subjects, and the differences between the groups should be normally distributed
• **Two Sampled (Independent) t-Test**	In situations where there are two separate samples, for example, the house prices in Mumbai v/s house prices in Delhi, and you have to check if the mean of both these samples is statistically significantly different not, then a two-sampled t-test can be used. It assumes that each sample's data distribution should be roughly normal, values should be continuous, the variance should be equal in both the samples, and they should be independent of each other
• **One-way Analysis of Variance**	An extension of two sampled t-tests is one-way ANOVA, where we compare more than two groups. Suppose someone asks you if that is ANOVA a parametric test, the answer to that is a definitive yes. ANOVA analyses the variance of the groups and requires the population distribution to be normal, variance to be homogeneous, and groups to be independent
• **Pearson's Coefficient of Correlation**	To understand the association between two continuous numeric variables, you can use a person's coefficient of correlation. It produces an 'r' value where a value closer to -1 and 1 indicates a strong negative and positive correlation respectively. A value close to 0 indicates no major correlation between the variables. A part of its assumption is that both the variables in question should be continuous.

Every parametric test has a nonparametric equivalent, which means for every type of problem that you have there'll be a test in both categories to help you out (Table 1).

Table 1. Parametric & Non-parametric tests with examples

Analysis Type	Example	Parametric test	Non-parametric test
Compare means b/w two independent groups	Is the mean systolic BP assigned to placebo patients different from the mean for treatment group patients?	Two-sample t-test	Wilcoxon rank-sum test/Mann Whitney U-test
Compare two quantitative measurements taken from the same individual	Was there a significant change in systolic BP b/w baseline and six-month follow up measurement in the treatment group?	Paired t-test	Wilcoxon signed-rank test
Compare means between three or more independent groups	If our experiment had three groups (e.g., placebo, new drug 1, new drug 2), then whether the mean systolic BP at baseline differed among the three groups?	Analysis of variance (ANOVA)	Kruskal-Wallis test
Estimate the degree of association b/w two quantitative variables	Is systolic BP associated with the patient's age?	Pearson coefficient of correlation	Spearman's rank correlation

The decision is dependent on other factors such as sample size, the type of data you have, what measure of central tendency best represents the data, etc. Now you can appreciate that in the example stated above, the statistical test to be applied would be Wilcoxon rank sum test (non-parametric test) and not Unpaired 't' test as the female sample dataset is not normally distributed. Applying this logic, the p-value would be 0.63 which is not significant!

References

1. Rosner, B. (2000). Fundamentals of Biostatistics, California: Duxbury Press.
2. Motulsky, H. (1995). Intuitive Biostatistics, New York: Oxford University Press.

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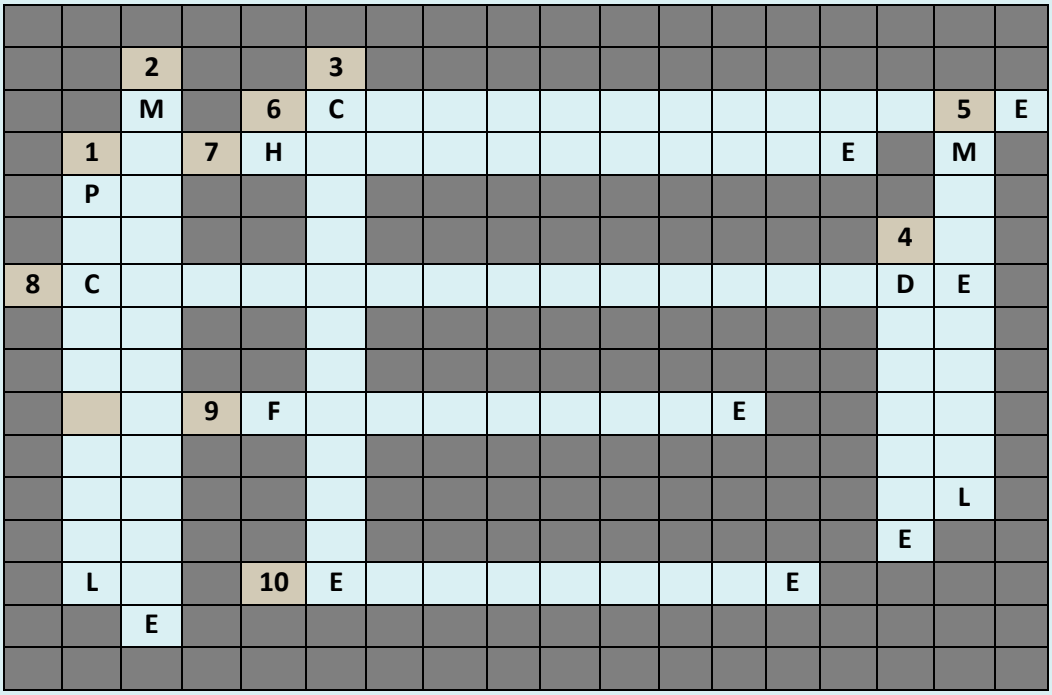

Downward	**Across**
1. Microtubule damaging drug used as an anticancer drug (*10*) 2. Semisynthetic ergot derivative with potent 5-HT receptor blocking action (*13*) 3. Calcineurin inhibitor (*12*) 4. Diamino diphenyl sulfone used in treatment of leprosy (*7*) 5. Progestin hormone used in chemotherapy of breast carcinoma (*9*)	6. Anti-seizure drug, also used in trigeminal neuralgias (*13*) 7. Direct vasodilator used in management of hypertension *(11)* 8. Alkylating agent used in regimens of cancer chemotherapy *(16)* 9. Cytotoxic antineoplastic drug used to manage and treat lymphoma, sarcomas etc. *(9)* 10. Antiarrhythmic drug acts by blocking voltage-gated sodium channels *(9)*

Answer to the Crossword Puzzle is given below:

Downward

1. Paclitaxel 2. Methylsergide 3. Cyclosporine 4. Dapsone 5. Merestrol

Across

6. Carbamazepine 7. Hydralazine 8. Cyclophosphamide 9. Fosfamide 10. Encainide